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Studies on the Mode of Survival of *Diplocarpon mali* Causing Premature Leaf Fall of Apple

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A B S T R A C T

Premature leaf fall is one of the most important diseases of apple particularly in Himachal Pradesh. Present studies were carried out with the objectives to study the mode of survival of *Diplocarpon mali* in the infected leaf litter, to study the effect of time of leaf fall, cultivar and environment on the perennation of *Diplocarpon mali*. Acervuli of *Marssonina coronaria* were found to occur in all the leaf samples while conidia formation started in or after May ranging from $1.2x10^3$ to $5.8x10^3$ conidia per ml at all the locations but its ascosporic stage and apothecia were not observed in any of the leaf samples. Leaves collected on September 28, 2013 produced the highest number of conidia ($5x10^3$ conidia/ml) in the summer and the conidial production started from pea stage of apple bud development and the maximum production of primary inoculum was observed at pre-harvest stage ($9.2x10^3$ conidia/ml). Maximum number of conidia was caught on the greased slides on 7th August 2014, when the temperature was 19ºC with ample rainfall and relative humidity above 85 per cent in the preceding 24 hours. Also, rainfall and relative humidity were significantly and positively correlated with number of conidia released in the apple orchard, whereas, the maximum temperature showed a negative correlation. Among the test apple cultivars, Golden Delicious produced maximum number of conidia in the overwintered leaf litter followed by Royal Delicious, Red Gold, Rich-a-Red, Scarlet Gala and Tydeman's Early Worcester and Granny Smith respectively.

Introduction

Apple cultivation has become the backbone of the economy of the hilly farmers in Himachal Pradesh, where the land is otherwise not considered ideal for traditional agriculture. Apple has become a leading commercial fruit crop being cultivated over 1,06,230 hectares with annual production of 4,12,400 metric tonnes and productivity is 3.9 MT per hectare in this state (NHB, 2012-13). Apart from its extensive cultivation, the fruit productivity of apple is not increasing accordingly in Himachal Pradesh. The productivity is quite lower in comparison to advanced apple growing countries of the world. The reasons for low apple productivity could be many but one of the reasons is the attack by a number of foliar, stem and root diseases during its growth period viz., apple scab, powdery mildew, root rot, brown rot, Alternaria blotch etc. Among them Marssonina blotch caused by

Diplocarpon mali Harada and Sawamura (Anamorph: *Marssonina coronaria* (Ell. & J.J. Davis) J.J. Davis) has lately gained significance due to its occurrence year after year over a vast area and is regarded as the cause of premature leaf fall of apple in Himachal Pradesh. This disease which was first recorded in the year 1992 in some orchards of Kotkhai area in Himachal Pradesh (Sharma *et al.,* 2004) is now affecting more than 90 per cent of orchards. After its first appearance sometimes ago, Marssonina blotch appeared in epiphytotic form in the state in 1996 and threatened the apple cultivation by causing premature leaf fall (Sharma and Kaul, 2000; Sharma, 2001). It was so severe in some orchards that no foliage was left on the trees by the harvest time (Sharma and Gautam, 1997).The disease symptoms appear both on leaves and fruits. Leaf spots usually develop on upper surface of mature leaves and are 5-7 mm in diameter, grayish brown and often tinged purple at the periphery. Small black acervuli are often visible on the surface. When lesions are numerous, they coalesce and surrounding areas turn chlorotic followed by defoliation. In Himachal Pradesh, symptoms appear after heavy rains in June-July as sudden yellowing of leaves showing brown to dark brown circular spots which often coalesce to form blotches. The pathogen first attacks the lower portion of the tree and fruits are commonly seen hanging from the defoliated branches, therefore this disease was commonly designated as "premature leaf fall" (Sharma and Gautam, 1997; Sharma, 1999). The fungus *Diplocarpon mali* primarily infects apple leaves. Ascospores produced in apothecia on overwintered leaves are believed to be the inoculum responsible for primary infections (Harada *et al.,* 1974). However, this pathogen is reported to perennate through conidia formed in the overwintering leaf litter in Himachal Pradesh (Sharma, 2004). Control is based on the use of protectant or eradicant fungicidal sprays to prevent the primary disease cycle. Nevertheless, present knowledge on the information about the disease and its perpetuation in the nature is still inadequate in India. Neither, any work has been done on its perennation in other countries. Therefore, the present investigation was carried out to study the mode of survival/ perennation of *Diplocarpon mali* in the infected leaf litter in the orchards of Himachal Pradesh and the effect of time of leaf fall, cultivar and environment on the survival/ perennation of *Diplocarpon mali* in nature.

Materials and Methods

Perpetuation of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in the infected apple leaf litter

Infected apple leaf litter of *Marssonina coronaria* was collected at different phenological stages of apple during 2013-14 from five locations of Shimla district and they were brought to the laboratory and analyzed microscopically for the development of primary inoculum. Dry apple leaves were placed in the hot boiling water for five minutes and were further wiped in the folds of blotting paper to make them pliable. Thin sections of the infected leaves showing pathogen fruiting bodies were cut and taken in a drop of lactophenol solution, were further viewed under a trinocular microscope. Observations on the formation of new crops of conidia/ ascospores were recorded. The primary inoculum (ascospores/conidia) load was also assessed by water extraction method from five different locations, i.e. Kotkhai, Rohroo, Thanedhar, Sarahan and Kullu (Gupta and Lele, 1980).

Ascospore productivity and pathogenicity test

Spore productivity was recorded by water extraction method of Gupta and Lele (1980).

Ten gram leaves in each replication were immersed in 100 ml of distilled water and kept for 4 hours at room temperature and afterwards thoroughly stirred in shaker for 30 minutes.

The content was decanted through a nylon mesh cloth and the suspension was centrifuged at 2800 rpm for 15 minutes. The pellet was suspended in 1 ml distilled water and number of conidia was estimated as the mean of five haemocytometer readings. Pathogenicity was proved by inoculating the potted apple plant leaves of Royal Delicious cultivar with *Marssonina coronaria* conidial suspension prepared from the above pellets and diluted to a concentration of $5x10^4$ spores/ml standardized by a haemocytometer.

An infection period of 12 hours was provided by maintaining 100 per cent humidity overnight by covering individual potted plants by perforated plastic bags of appropriate size. The potted plants were then incubated for 12 days when first symptoms were observed at an ambient temperature ranging from 20-25ºC in a polyhouse.

The observations were recorded on the symptom development, and re-isolation of the microorganisms was also ascertained on a modified sucrose-asparagine medium.

Studies on effect of time of leaf fall on the production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in apple orchard

Fallen apple leaves (100 number) infected with Marssonina blotch from unsprayed orchard at Regional Horticultural Research and Training Station (RHRTS), Mashobra were collected, placed in nylon mesh bags and left in the orchards for overwintering towards the end of 2013. Leaves were collected four times on 8th September, 28thSeptember, 18th October and 8th November, 2013 and were placed in the specially prepared mesh bags for overwintering.

Production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in apple orchards at different phenological stages

Unsprayed overwintered Marssonina blotch infected leaves of Royal Delicious cultivar were collected earlier from the orchard floor of Regional Horticultural Research and Training Station (RHRTS), Mashobra were brought to laboratory and analyzed microscopically for the production and productivity of primary inoculum in the form of conidia/ ascospores at different phenological stages of apple development. The stages were green tip, pink bud, petal fall, fruit set (pea size), fruit development (walnut size) and pre harvest stage.

Their viability was adjudged by inoculating apple potted plants and data/ observations on the disease development and severity also recorded (Sharma *et al.,* 2005). Observations were recorded for the production of conidia/ ascospores under microscope and number of conidia per ml were calculated by taking five haemocytometer readings.

In the month of April, one fourth of leaves were brought to laboratory, kept at room temperature and observed for production of primary inoculum in the form of conidial/ ascosporic stage under microscope by using haemocytometer. Remaining three fourth part of leaves were brought to laboratory in the month of May, June and July, 2014 for observations in the same manner as mentioned above. Observations were recorded for the production of conidia/ ascospores under microscope and number of conidia per ml as calculated by taking five haemocytometer readings.

Effect of environmental factors on the spore discharge of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in apple orchard at RHRTS, Mashobra

In order to assess the amount of primary inoculum released in the orchard, greased slides were used to trap the spores of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*). The greasing was done with the white petroleum jelly.

The smeared slides were suspended in the air at a height of 45 cm facing the over-wintering leaves for 3 hours interval from 6 a.m. to 9 p.m. The observations were recorded first on 7th June and repeated at fortnightly intervals till the fruit harvest. The greased slides were observed microscopically and average number of spores per ten slides was worked out. The total slide area was divided into four segments and number of spores was counted in each of them.

Effect of cultivar on the production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) causing premature leaf fall in apple

Marssonina blotch infected leaves of different apple cultivars viz., Royal Delicious, Golden Delicious, Red Gold, Tydeman's Early Worcester, Rich-a-Red, Granny Smith and Scarlet Gala were collected in the autumn on 28th September, 2013 and placed on the orchard floor in nylon mesh bags for overwintering in the apple orchard of Regional Horticultural Research and Training Station (RHRTS), Mashobra, Distt. Shimla, Himachal Pradesh. The leaves of each cultivar were brought to the laboratory four times viz., April, May, June and July, 2014 and observed for production of primary inoculum in the form of conidial/ ascosporic stage under microscope and spore productivity was calculated based on 5 haemocytometer reading. Thus, observations were recorded in the form of number of conidia per ml.

Results and Discussion

Perpetuation of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in the infected apple leaf litter

Microscopic examination of overwintered leaf litter revealed that new crops of conidia were formed in the pre-existing acervuli in the overwintered apple leaves in the months of June and July in Shimla and Kullu districts of Himachal Pradesh (Fig. A). The perfect state of *Marssonina coronaria* i.e. *Diplocarpon mali* was however not intercepted in any of the samples (Table 1). The primary inoculum (ascospores/conidia) load was also assessed by water extraction method from five different locations, i.e. Kotkhai, Rohroo, Thanedhar, Sarahan and Kullu.

Acervuli of *Marssonina coronaria* were found to occur in all the leaf samples while conidia formation started in or after May. No apothecia were observed in any of the leaf samples.

It is evident from the perusal of data in Table 2 that no ascosporic stage of *Marssonina coronaria* was found to occur in any of the leaf samples. No conidia production was recorded at different locations during the period of March and April. During May, $4.8x10^3$ and $2.4x10^3$ conidia per ml were formed at Kotkhai and Rohroo, respectively. However, fresh crops of conidia were formed in the pre-existing acervuli from June onwards, ranging from $1.2x10^3$ to $5.8x10^3$ conidia per ml at all the locations but $2.0x10^3$ to $5.0x10^3$ conidia per ml were formed during July at Thanedhar and Sarahan, respectively (Fig. B). The pathogenicity of the conidial inoculum produced in the leaf litter was proved as disease symptoms over recorded in

the inoculated apple plants after 12 days (Fig. C). The secondary inoculum was also produced as typical conidia in the infected leaves. Apple leaves infected with Marssonina blotch were collected on different dates of leaf fall and kept in the field for overwintering. They were analyzed for production of primary inoculum in the following spring.

The data on the production of primary inoculum presented in Table 3 revealed that leaf fall time had a marked influence on their productivity of primary inoculum i.e. conidia in the overwintered leaf litter in apple orchard. There was a great variation in the production of primary inoculum in the leaves collected and observed on four different dates. Collected leaf samples were further analyzed for four consecutive months viz., April, May, June and July in 2014. It was found that leaves which were collected on September 28, 2013 produced the highest number of conidia ($5.0x10^3$ conidia/ml), followed by $3.8x10^3$ and $2.5x10^3$ conidia per ml collected on September 08 and October 18, 2013, respectively.

The productivity of primary inoculum was found to be the least ($1.9x10^3$ conidia/ml) in the leaves which were collected in the month of November. The data also indicated that as number of days increased from April to July, there was a corresponding increase in production of primary inoculum of *Marssonina coronaria* irrespective of the time of leaf fall in the apple orchard.

Production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in apple orchard at different phenological stages

Overwintered Marssonina blotch infected apple leaves were collected from the orchard floor and brought to the laboratory at different stages of apple bud development, starting from green tip up to pre-harvest stage.

Overwintered apple leaves collected from apple orchard of Regional Horticultural Research & Training Station (RHRTS), Mashobra at different phenological stages were examined microscopically for productivity of conidia/ ascospores and data so obtained are presented in Table 4 which revealed that no conidial or ascosporic stage was found at green tip, pink bud and petal fall stages of apple bud development.

Initiation of primary inoculum (conidia) production began from pea size stage and increased subsequently with the advancement of time. Maximum production of primary inoculum was observed at pre-harvest stage ($9.2x10^3$ conidia/ml).No ascosporic stage was found at any of apple bud development stages in the present study.

Release of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) conidia in apple orchard at Regional Horticultural Research & Training Station (RHRTS), Mashobra during summer

Greased slides were used to catch the spore flights by air current on five different dates in the months of June, July and August in 2014. The slides were suspended in the apple orchard at a height of 45 cm from the ground floor for 3 hours from 6 a.m. to 9 p.m. to see the diurnal pattern of conidia release in the orchard. The slides were observed for the conidia trapped and data were analyzed.

The data on the number of ascospores and conidia recorded on different dates are presented in Table 5. The perusal of the data reveals that no ascospores of *Diplocarpon mali* were trapped on the slides, however, maximum number of conidia were trapped on 7th August 2014 followed by 23rd July, 7th July and 23rd June, 2014. The number of conidia trapped also increased with time from 7th June to 7th August 2014.

Table.1 Development of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in the overwintered leaf litter during 2013 season

Location	Formation of acervuli/conidia/apothecia														
	March			April			May			June			July		
	Ac	C	Ap	Ac	C	Ap	Ac	C	Ap	Ac	C	Ap	Ac	C	Ap
Kotkhai	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes	No	-	-	-
Rohroo	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes	No	-	-	-
Thanedhar	Yes	No	No	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes	No
Sarahan	Yes	No	No	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes	No
Kullu	Yes	No	No	Yes	No	No	Yes	No	No	Yes	Yes	No	-	-	-

Ac= Acervuli C= conidia Ap = Apothecia - = No observation

Table.2 Productivity of conidia of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in the overwintered leaf litter during 2013 season

Location	Number of conidia produced per ml				
	March	April	May	June	July
Kotkhai	Nil	Nil	$4.8x10^3$	$5.0x10^3$	-
Rohroo	Nil	Nil	$2.4x10^3$	$2.8x10^3$	-
Thanedhar	Nil	Nil	Nil	$5.8x10^3$	$5.0x10^3$
Sarahan	Nil	Nil	Nil	$1.2x10^3$	$2.0x10^3$
Kullu	Nil	Nil	Nil	$1.5x10^3$	-

Table.3 Effect of time of leaf fall on production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) causing premature leaf fall in apple

Sr. No.	Time of leaf fall (Date of collection)	Formation of conidia/ ascospores		Production of primary inoculum* (Number of conidia per ml)				
		Conidia	Ascospores	April	May	June	July	Mean
1.	Sept. 08, 2013	+	-	$2.0x10^3$	$2.8x10^3$	$4.0x10^3$	$6.4x10^3$	$3.8x10^3$
2.	Sept. 28, 2013	+	-	$2.8x10^3$	$3.6x10^3$	$5.2x10^3$	$8.4x10^3$	$5.0x10^3$
3.	Oct. 18, 2013	+	-	$1.2x10^3$	$2.4x10^3$	$2.8x10^3$	$3.6x10^3$	$2.5x10^3$
4.	Nov. 08, 2013	+	-	$0.8x10^3$	$1.6x10^3$	$2.0x10^3$	$3.2x10^3$	$1.9x10^3$

Table.4 Production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) in apple orchard at different phenological stages

Sr. No.	Phenological stage	Formation of conidia/ ascospores		Production of primary inoculum* (Number of conidia per ml)
		Conidia	Ascospores	
1.	Green tip	-	-	0
2.	Pink bud	-	-	0
3.	Petal fall	-	-	0
4.	Fruit set (Pea size)	+	-	$2.8x10^3$
5.	Fruit development (Walnut size)	+	-	$3.2x10^3$
6.	Fruit development (20 days after)	+	-	$6.0x10^3$
7.	Pre-harvest stage	+	-	$9.2x10^3$

Table.5 Release of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) conidia in apple orchard at RHRTS, Mashobra during summer

Sr. No.	Date of observation	Number of spores trapped per ten slides		Weather parameters				
				Temperature (°C)			R.H. (%)	Rainfall (mm)
		Ascospores	Conidia	Minimum	Maximum	Mean		
1.	June 07, 2014	-	0	15.8	27.4	21.6	57	0
2.	June 23, 2014	-	5	13.8	22.7	18.25	78	0.2
3.	July 07, 2014	-	11	12.5	23	17.75	63	0
4.	July 23, 2014	-	18	14.8	23.5	19.15	87	14.8
5.	Aug 07, 2014	-	23	15.2	23.5	19.35	91	47.4

Table.6 Correlation of number of *Diplocarpon mali* (Anamorph: *Marssonina coronaria)* conidia released in apple orchards with the environment factors during summer

Simple correlation coefficient					
Parameter	Min. Temp.	Max. Temp.	Avg. Temp.	R.H.	Rainfall
Number of conidia	0.012	-0.545	-0.348	0.823*	0.842*

* - Highly significant

Table.7 Effect of cultivar on the production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) causing premature leaf fall in apple during summer at RHRTS, Mashobra

Sr. No.	Apple cultivar	Formation of conidia/ ascospores		Production of primary inoculum* (Number of conidia per ml)				
		Conidia	Ascospore	April	May	June	July	Mean
1.	Royal Delicious	+	-	$2.8x10^3$	$3.2x10^3$	$6.0x10^3$	$9.2x10^3$	$5.3x10^3$
2.	Golden Delicious	+	-	$3.6x10^3$	$4.4x10^3$	$5.6x10^3$	$8.4x10^3$	$5.5x10^3$
3.	Red Gold	+	-	$2.0x10^3$	$2.4x10^3$	$4.0x10^3$	$7.6x10^3$	$4.0x10^3$
4.	Tydeman's Early Worcester	+	-	$1.2x10^3$	$1.6x10^3$	$1.6x10^3$	$3.6x10^3$	$2.0x10^3$
5.	Rich-a-Red	+	-	$1.2x10^3$	$1.6x10^3$	$3.2x10^3$	$6.4x10^3$	$3.1x10^3$
6.	Granny Smith	+	-	$0.8x10^3$	$0.8x10^3$	$1.2x10^3$	$2.4x10^3$	$1.3x10^3$
7.	Scarlet Gala	+	-	$1.6x10^3$	$1.2x10^3$	$2.8x10^3$	$5.6x10^3$	$2.8x10^3$

*Based on five haemocytometer reading

The relationship of weather data of preceding 24 hours showed that heavier conidial discharge was related to optimum temperature at 19°C, good amount of rainfall or 85 per cent or above relative humidity. A large number of conidia and the spores of other fungi were also observed as contaminants on these slides. Apart from others, maximum temperature showed negative correlation with number of *Marssonina coronaria* conidia released in the apple orchard whereas relative humidity and rainfall showed a highly significant and positive correlation with the release of conidia under orchard conditions.

Effect of cultivar on the production of primary inoculum of *Diplocarpon mali* (Anamorph: *Marssonina coronaria*) causing premature leaf fall in apple during summer at Regional Horticultural Research & Training Station (RHRTS), Mashobra

It is evident from the data presented in Table 7 that all cultivars showed a positive response for formation of conidia in the leaves which were kept in apple orchard for overwintering. It is also clear from the mean data that Golden Delicious cultivar produced maximum number of conidia ($5.5x10^3$ conidia/ml) followed by Royal Delicious ($5.3x10^3$ conidia/ml), Red Gold ($4.0x10^3$ conidia/ml), Rich-a-Red ($3.1x10^3$ conidia/ml), Scarlet Gala ($2.8x10^3$ conidia/ml) and Tydeman's Early Worcester ($2.0x10^3$ conidia/ml). Whereas Granny Smithcultivar was found to produce minimum amount of primary inoculum in the form of conidia ($1.3x10^3$ conidia/ml) among all the test cultivars. It was also noticed that production of primary conidial inoculum increased subsequently with the advancement of time in all the test cultivars. Ascosporic stage was not formed in any of the overwintered leaves again in this experiment also.

In the present studies, the conidial production of *Marssonina coronaria* was found maximum in apple leaves which fell earlier in the autumn. The leaves collected on September 28, 2013 produced the highest number of conidia followed by those collected on September 08 and October 18, 2013, whereas, the productivity of primary inoculum was found to be minimum in the leaves collected in the month of November. This shows that the most important inoculum for the primary infection comes from the leaves fallen in the months of September and October. Similarly, Wilson (1928) observed that leaf fall had a great influence on the time of ascospores maturity in the following spring and delayed leaf fall minimized the infection of young leaves. But James and Sutton (1982) found no effect of time of leaf fall and cultivars on ascospores maturity. The results also indicated that as number of days increased from April to July, there was a corresponding increase in production of primary inoculum of *Marssonina coronaria* irrespective of the time of leaf fall in the apple orchard.

Overwintered apple leaves collected from the experimental orchard of Regional Horticultural Research and Training Station (RHRTS), Mashobra at different phenological stages and were also examined for the production of primary inoculum. These studies revealed that conidia or ascospores were not formed in the overwintering leaf litter at green tip, pink bud and petal fall stages of apple bud development. The initiation of conidial production began from pea size stage and it was maximum at pre-harvest and fruit development stages during 2014 apple season. These studies also indicated that conidial production increased subsequently with the advancement of time and the release of conidia was observed on 7th June, 2014 which increased with increase in time and the release was maximum on 7th August, 2014. The conidia of *Marssonina coronaria* produced in pre-existing acervuli which resulted in fresh infection, and their number also increased with the passage of time. The environmental parameters were 19^{o}C temperature with 47.4 mm rainfall and above 90 per cent relative humidity on 6th August, 2014. There is no earlier study with regard to *Marssonina coronaria* in the literature; however, ascospores discharge of *Venturiain aequalis* has been reported from the overwintered leaf litter of apple from green tip with a peak at full bloom and petal fall stages in Himachal Pradesh (Nath, 1983; Sharma and Gupta, 1995).

A temperature of 17-22^{o}C was also found best for ascospores discharge by Kulibaba and Cherepkova (1970) in U.S.S.R. In addition to temperature, Brook (1969) described humid and warm weather to be conducive for the spore discharge. High rainfall has also been associated with increased ascospores discharge which directly affects disease severity (Bremer, 1924; Osmum, 1922; Kuthe, 1962; Borecki and Cichosz, 1961) These results are also inconformity with the results obtained by Sharma *et al.,* (2005) who reported that Marssonina blotch disease was favoured by moderate temperature (20-22^{o}C) and high relative humidity following rains in the summer. Gao-Yue *et al.,* (2011) reported that the overwintered fungus, *Diplocarpon mali* produced pseudoconidia at temperature ranging from 0 to 30^{o}C with an optimum at 15.5^{o}C. Being wetted or high relative humidity environment was necessary for overwintered fungus to produce pseudoconidia. After being wetted or putting into environment with relative humidity over 97 per cent, the fungus formed abundant pseudoconidia within 6 h. They also indicated that few apothecia were formed in early March, whereas their frequency rose to 0.34 per cent in later part of this month, which was 27 per cent in late May on the overwintered leaves basis.

There was a definite relationship between Marssonina blotch vis-a-vis rainfall, temperature and relative humidity. Rainfall, relative humidity and minimum temperature were positively correlated with number of *Marssonina coronaria* conidia released in the apple orchard during summer. Maximum temperature, however, was negatively correlated with number of conidia of premature leaf fall pathogen. There was a highly significant correlation of rainfall and relative humidity with the number of conidia released in the apple orchard. These results corroborate the findings of Sharma (2003) who reported that the development of disease was positively correlated with relative humidity and rainfall. Sharma *et al.,* (2004) reported that this disease was favoured by high rainfall and moderate temperature ranging from 20-22^{o}C during the fruit development stages of apple. Similarly, Thakur *et al.,* (2005) reported that the prevalence of blotch disease was influenced by leaf ageing followed by continuous average temperatures above 20^{o}C and 70 per cent relative humidity for 5-6 days which induced the yellowing and defoliation process. These results are also in line with the results obtained by Sharma *et al.,* (2005) who reported that Marssonina blotch disease was favoured by moderate temperature (20-22^{o}C) and high relative humidity following rains in the summer.

Inoculum potential is an aspect which can prove helpful in forecasting the diseases as it is one of the important factors indicating the susceptibility of different cultivars. In the present study, Golden Delicious was found to be the most susceptible cultivar producing maximum amount of primary inoculum followed by Royal Delicious, Red Gold, Rich-a-Red, Scarlet Gala, Tydeman's Early Worcester and Granny Smith. In Himachal Pradesh, the Delicious group comprises the main bulk of the cultivars and thus quite a high amount of inoculum produced by these cultivars poses a threat to the apple industry time and again. These findings are also more or less in line with those of Sharma and Gautam (1997) who reported that Golden Delicious, Royal Delicious, Red Gold and Fuji were found highly susceptible to premature leaf fall in apple. Earlier, different workers have reported some cultivars like Tydeman's Early Worcester and Granny Smith as moderately resistant to resistant to this disease (Sharma and Verma, 1999; Bala, 1999). These results are in conformity with those of Gupta (1980) who observed the susceptible cultivars produced more inoculum quantum of *Venturiain aequalis* causing scab in apple. Sharma *et al.,* (2011) also reported that Marssonina leaf blotch (*Marssonina coronaria*) did not develop on five cultivars viz., Democrat, Maharaja Chunth, Mutsu, Golden Spur and Granny Smith.

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