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Predicting Potential Ascospore Dose of *Venturia inaequalis* (Cks) Wint in Farmers Apple Orchards in Central Himalayas of India

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ABSTRACT

Experiments were conducted to forecast the Potential Ascospore Dose (PAD) of the apple scab, caused by *Venturia inaequalis* (Cks.) Wint. (anamorph: *Spilocaea pomi* Fr.), was evaluated in Gangotri valley over 15 years (1994-2012) at four different place. In India, the disease causes significant economic losses in the states of Jammu and Kashmir and Himachal Pradesh each season and has been prevalent in Uttarakhand hills, the 3rd largest apple growing state in the country. Yield losses during epidemic years in 1996 and 2008 in the region went up to 70%. The PAD has great impact on disease development in the following season. It varied between 645-373421 in various orchards of Gangotri valley. The PAD value was low during 1999-2001 (645-1894 ascospore/m^2) and medium during 2002-2006 (2108-29685 ascospore/m^2) due to the fact that the springs were early, dry and unfavorable for ascospore maturation. In 1996 and 2008, the PAD values were high because of the favourable weather conditions and increase of inoculum accumulation. The scabbed lesion and leaf litter density were approximately more than twice in Gangotri fruit belt in 1996 and 2008. For this reason, reduction of primary inoculum sources played very important role in the improvement of scab management of apple in Uttarakhand Himalayas.

Key words: Apple, pseudothecia, ascospore, potential ascospore dose

INTRODUCTION

Apple scab caused by *Venturia inaequalis* (Cooke.) Winter (anamorph: *Spilocaea pomi* Fr.), is one of the most serious diseases of apple worldwide. In Uttarakhand, this disease had remained confined to the Gangotri valley for the last twenty years. The disease is now well established and could cause almost total crop loss during most years, if not controlled (Singh *et al*., 2010). It is one of the rare diseases, wherein the disease level is largely determined by the amount of inoculum at the beginning of the growing season. According to Singh and Kumar (2009), the availability of inoculum in the orchard greatly influenced by the pattern of disease level of the previous season. Yield losses during epidemic years can go up to 70% or even more. During 1996, an estimated Rs. 40 lakhs worth of apple fruit was wasted due to scab disease in Gangotri valley. During this year Uttar Pradesh Govt. had taken decision to purchase all the fruits at Rs. 2.00 kg^{-1} and dumped at other places. During 2008, scab made nearly 23% of the apple crop unfit for either market consumption or processing (Singh and Kumar, 1999, 2004, 2007, 2008; Singh *et al*., 2010). Over 60% of the area under sweet varieties is now engulfed by apple scab.

The concept of Potential Ascospore Dose (PAD) introduced by MacHardy and Jeger (1983), is a useful tool for predicting the total amount of inoculum in an orchard. Gadoury and MacHardy (1986) have worked on different inoculum levels (PAD) under Norwegian conditions, where reduced spraying can be tolerated. At present, the PAD technique is the most frequently used method in Western countries for best forecast of ascospore production in an orchard in order to predict the danger of epidemic in early spring (MacHardy and Sutton, 1997; Stensvand and Amundsen, 1997; Holb and Heijne, 2002; Singh *et al*., 2010; Charest *et al*., 2002; Singh and Kumar, 2009) but refinements in the present method for determining PAD may be needed, as PAD is likely to be area specific. Estimation of potential ascospore dose is useful when comparing management strategies or control treatments in several orchards. Ultimately, improve scab management efficiency with reduced fungicides use, more reliable scab warning system would be imperative.

Weather conditions, especially rainfall, duration of leaf wetness and temperature vary at different altitudes in the Himalayan region, where apple is cultivated. Therefore, understanding the timing and intensity of environmental conditions that trigger ascospore release may provide useful information to growers, who use fungicides to protect their trees from new infection. Thus, the objective of this study was to determine potential ascospore dose from farmers orchard at three different locations in Uttarakhand Himalayas.

MATERIALS AND METHODS

Five farmers orchards were selected at three different places of Gangotri valley and in each orchard five apple trees were marked for sampling in the month of October, 1994-2012. Prior to onset of leaf fall in autumn, the amount of scab on the foliage was assessed by number of scab lesions per leaves on 30 terminal shoots on 15 randomly selected trees in each orchards. Leaf area per terminal shoot was calculated by measuring leaf size on 30 randomly selected terminal shoots per tree for each orchard with an electronic leaf area meter (CID, USA). All the established scab lesions were counted in the leaves of each sampling replicate of m^{-2} at leaf fall. The ten random zones (each 1 m^2 area) with well spread apple leaves were collected and weighed for determination of each level of leaf litter. The average weight of 10 marked zones was assumed to represent the level of leaf litter m^{-2} ground floor under that particular sampling tree. Number of leaves equivalent to average weight determined m^{-2} ground floor under each sampling tree were sealed in a nylon mesh bag and kept for overwintering under natural conditions. Subsequent to this, ten discs (5 mm) from these leaves were randomly, cut with the help of cork borer and these were subjected to tissue clearing treatment. Such cleared discs were observed under microscope for counting the number of pseudothecial initials. After 60, 75, 90 and 130 days of overwintering of leaves, 25 pseudothecia, randomly picked up from below the cuticle of 10 discs in each lot were gently pressed and observed under the microscope for the number of asci with hyaline and colored ascospores.

Pseudothecial density was observed from the overwintering leaves of each lesion after 60 days of sampling and their surface areas was measured with the help of a leaf area meter. Leaf lesion discs (5 mm) were observed under microscope for counting total number of pseudothecia having developed on 10 such discs and same were divided by ten. The pseudothecial density, number of pseudothecia per lesion was multiplied with lesion density in m^2 leaf area. Data on two different ascal stage i.e., asci with immature or hyaline ascospores and asci with mature or colored ascospores, were made after five month of overwintering of leaves. Each time, 25 discs with scab lesions were treated with chloral hydrate and mounted on lacto phenol. The pseudothecia were crushed gently under the microscope for taking observation on the proportion of asci with immature and mature ascospores.

Ascospore density was the product of the number of ascospores/ascus and the proportions of asci with mature ascospores/pseudothecium. The latter was set to 137 asci/ascocarp with a standard error of 9.2. The ascospore productivity per fertile lesion was the product of pseudothecia per fertile lesion (26.3)×asci/pseudothecium (137)×ascospores/ascus (8). The amount of scab on the foliage was computed by dividing the number of lesion counted on leaves of 200 shoots by a mean total leaf area (assuming a mean leaf area per terminal shoot of 347 cm^2).

PAD gives the estimated production of ascospores m^{-2} of orchard floor and the estimated number of ascospores produced per fertile lesion and was calculated by the following method given by MacHardy (1996):

$$PAD = LD \times LLD \times PD \times AD \times n$$

The Potential Ascospore Dose (PAD) for each orchard was the product of the lesion density (number of lesions on leaves per square meter of orchard floor at leaf fall), leaf litter density (proportion of the orchard floor covered by leaf litter at bud break), pseudothecial density (number of mature pseudothecia/visible lesion multiplied by a lesion fertility factor), ascus density (number of asci/pseudothecium) and number of ascospores/ascus. The PAD was used to forecast the onset of apple scab epidemics in the orchard. Fungicides were applied according to a schedule. Most sprayings were based on predictions from electronic scab warning devices. Mill's table was adopted for calculating infection periods for the appearance of primary scab. When spraying after an infection period, EBI fungicides were used. Only unsprayed orchards of previous season showing high disease severity at harvest were considered for PAD estimation in current season.

The delay of an apple scab epidemic from green tip in number of days Δt was calculated from the PAD. The PAD is used to forecast the onset of apple scab epidemics in commercial orchard. Van der Plank (1963) first described the general mathematical relationship between difference in the amount of overwintering inoculums and the early progress of an apple scab epidemic can be expressed mathematically as,

$$\Delta t = l/r \text{ In } Xo/Xos$$

Where Δt = the delay of an apple scab epidemic or the shift in time of a disease progress curve due to difference in the amount of overwintering inoculums, r = the rate of infection and Xo/ Xos = the ratio of the difference in inoculums between tow orchards.

RESULTS

The leaves having lower number (32-72) of lesions/m^2 leaf area yielded higher number of pseudothecial initials (402-416), while there were only 64-96 initials per ten discs at the higher number of lesions per m^2 leaf area (1045-1282). The linear regression equation was plotted between years. Figure 1 shows 66% accuracy relationship between lesion density and pseudothecial initials. The pseudothecia filled up with pseudoparaphyses after 30 days the development of pseudothecial initials did reveal the 64% accuracy in between these two parameters under study. Developed pseudothecia were collected after four month from selected orchards, in which asci had started appearing. Figure 1 shows, 69% accuracy relationship between lesion density and developing pseudothecia filled, with asci during overwintering period. The linear regression equation (y = 264.8-0.148x) also demonstrated a positive correlation between the lesion density and developing pseudothecia. The ascal population was influenced by lesion density and there was 86% accuracy observed during most of the years (Fig. 1). The regression lines (y = 134.8-0.093x) also reflected the positive slop between these two parameters. Similar accuracy (96%) was also exhibited between numbers of asci with hyaline ascospores. The regression line (y = 109.6-0.069x) as shown in Fig. 1, further showed the same trend. The leaf lesion density was also correlated with the production of mature asci, which on being multiplied with 8 as number of ascospores per ascus revealed the potential ascospore dose available in the orchard for discharge and causing infection. The regression line (y = 120.6-0.082x) reflected the 91% accuracy in between lesion density and production of mature asci. Finally, the data showed that pseudothecial development and ascospore maturity were positively correlated to lesion density.

The pseudothecia were crushed gently under the microscope and observed. It was found that the level of pseudothecial density established during the overwintering period with the population of asci having immature and mature ascospores showed 97% accuracy to predict the ascospore development. The linear regression equation plotted in Fig. 2, revealed that the population of asci with immature and mature ascospores had positive slopes with the levels of pseudothecial densities.

The development of various stages of pseudothecia starting from the initiation to their maturity was also observed in relation to the leaf litter density (Fig. 3). The lower levels of

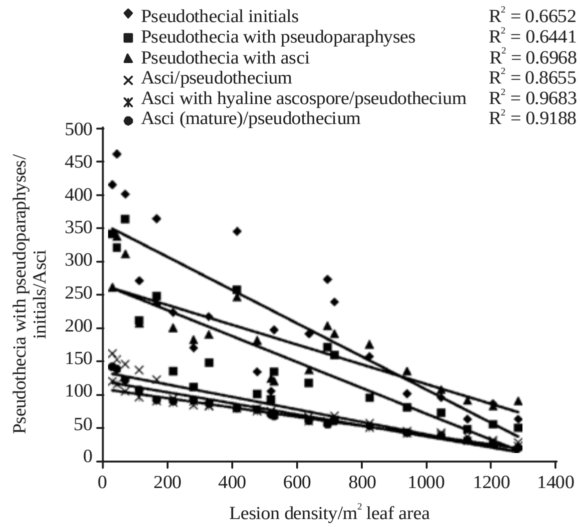

Fig. 1: Influence of leaf lesion density on the development of pseudothecia, asci and ascospores

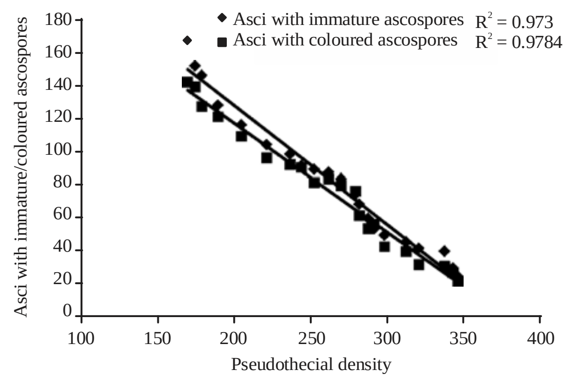

Fig. 2: Relationship of pseudothecial density with the development of asci having immature and mature ascospores

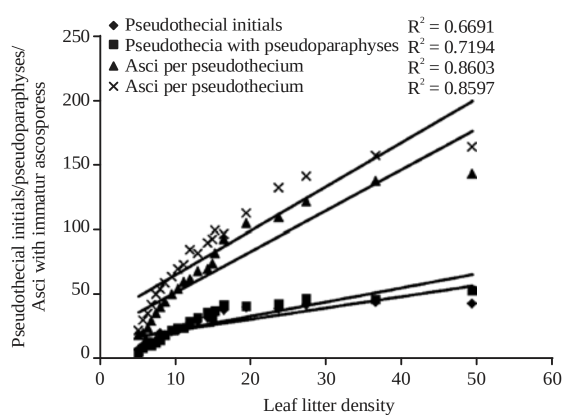

Fig. 3: Effect of leaf litter density on the development of pseudothecia and asci having immature and mature ascospores

leaf litter density (5.2) yielded a lower number of pseudothecial initials (6) while, there were as much as 42 initials per ten discs at higher levels of leaf litter m^{-2} orchard floor (49.28). The coefficient of determination (R^2) value in each equation was established to signify the% accuracy of the equation, which was established to predict the pseudothecial maturity. The prediction equation for leaf litter density with the development of pseudothecial initials showed 67% accuracy to predict the initiation of pseudothecia in the last week of November in Gangotri valley. The linear regression equation (y = 11.91+0.890x) were plotted in Fig. 3, reflected the positive slope between these components. The same pattern of relationship (71% accuracy) was also observed between developing pseudothecia filled up with pseudoparaphyses after one month of the development of pseudothecial initials (Fig. 3). The number of asci with immature and mature ascospores were observed after 130 days of sampling. The prediction equation (y = 18.56+3.20x, y = 29.81+3.445x) between leaf litter density and number of asci with immature and mature ascospores showed 86% accuracy to predict the immature and mature ascospores. The regression lines as drawn in Fig. 3 also reflected the positive slope between these two parameters. The mature ascospores per pseudothecium available on the orchard floor at the levels of 49.28 g leaf litter density was 1246, as each ascus would contain eight ascospores.

The farmers' orchards, spread along the Ganges in Gangotri fruit belt harboring mostly Delicious group of apple, experience chronic cycles of scab development each season leading to heavy fruit losses. The scab development was monitored over 15 years in farmers' fields from 1994 through 2012 and PAD was found to range from 645 to 3, 87,782. Figure 4 shows the components of estimated PAD in comparison with each other at farmer's orchards in Gangotri valley. Environmental conditions were very similar at different experimental sites in Gangotri valley (Dharali, Harsil and Jhalla). As is evident from Fig. 4, PAD was below 1000 (1999-2005) and there was no adverse effect of delaying the first spray after the petal fall stage. At this time, the proportion of ascospore that was mature was very low and the amount of foliage infection was also low. Two to three sprays of EBI fungicide at the end of primary infection inoculum season in mid or late June had no effect on scab development. As is evident from Fig. 5, PAD was low and the first Mills infection period for the season occurred 13 days after petal fall. Fifteen to 18 infection periods were recorded from last week of May to September. Three sprays during this period gave good control of disease compared to the unsprayed, only one spray reduced scab on the leaves to a great extent and eliminated scab on the fruits during 1999-2001.

DISCUSSION

At present, PAD is a useful tool to forecast the total amount of inoculum in an orchard and has been shown to effectively improve apple scab management (MacHardy *et al*., 1993). We studied different inoculum levels under Central Himalayan conditions, where reduced spray program could be used after petal fall for the management of scab. The slow buildup of matured ascospores during lag phase (sigmoid

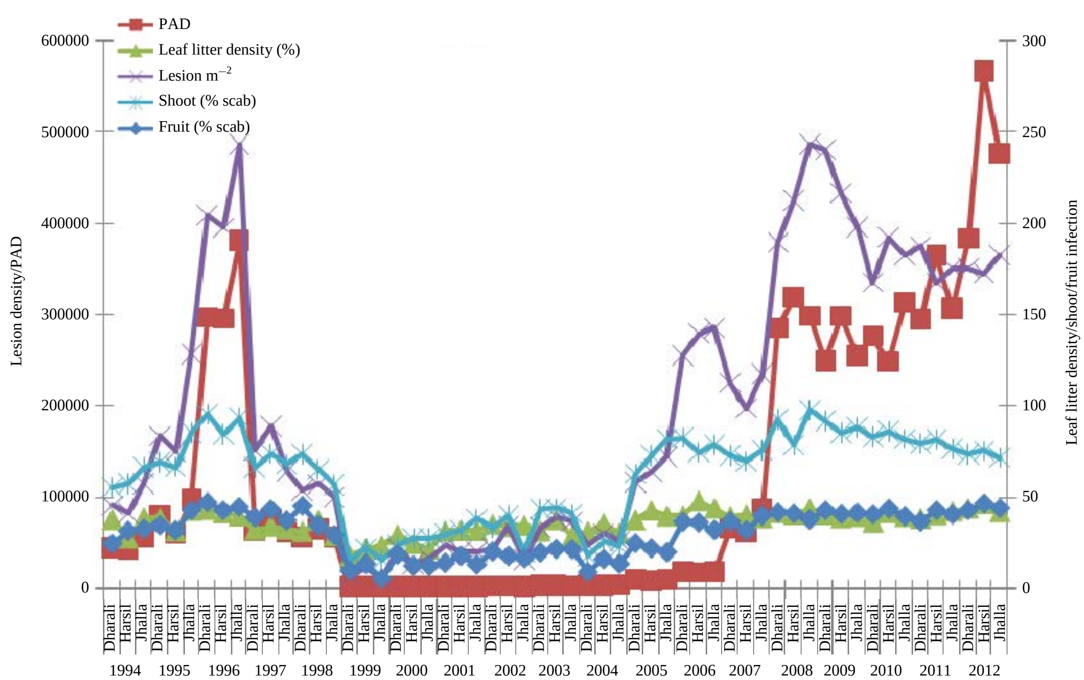

Fig. 4: Compression of different component variable of PAD at farmer's orchards in Gangotri valley

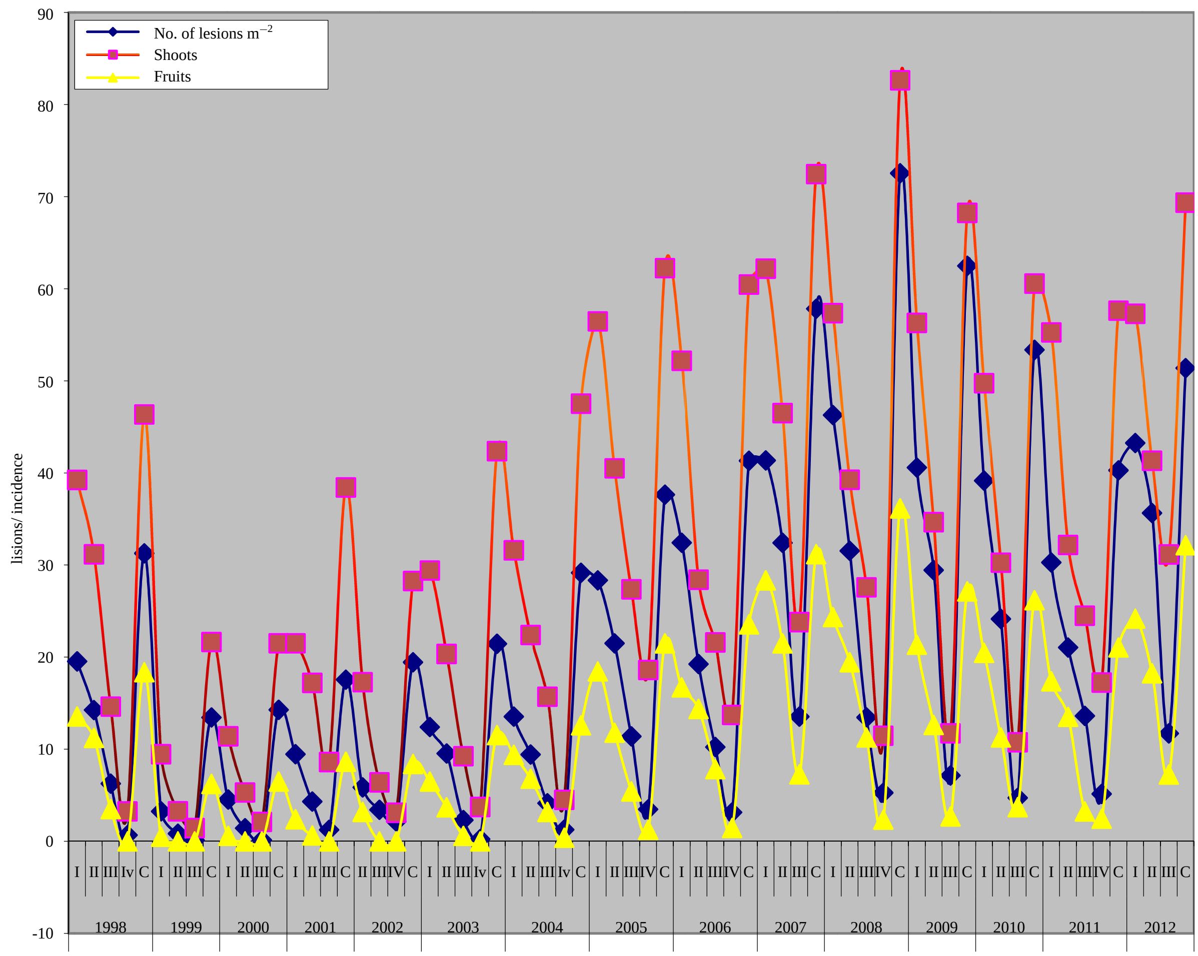

Fig. 5: Scab incidence at harvesting stage in Gangotri valley of Uttarakhand (Chemicals were sprayed after estimation of potential ascospore dose)

curve) could be relatively easy to control with fungicides (post infection strategy). At the time of accelerated phase, the crop is potentially at a high risk, if proper control is not exercised. Protective or post infection strategies could be employed but a protective schedule is usually preferred, especially if scab was not managed well during the previous year.

The PAD, which has great impact on disease development in the following season varied from 645-3, 87,782 in the various orchard of Gangotri fruit belt. The PAD value was low during 1999-2001 (645-1894 ascospore/m^2) and medium during 2002-2006 (2108 -29685 ascospore/m^2) due to the fact that the springs were early, dry and unfavorable for ascospores maturation. In 1996 and 2008, the PAD values were high because of the favourable weather conditions and increase of inoculum accumulation. The scabbed lesion and leaf litter density were approximately more than double in Gangotri fruit belt in between 1994-1996 and 2007-2012. This was probably due to, reason that winter and early springs are more mild and rainy in the Himalayan range of Uttarakhand hills. The susceptible cultivars (Red, Royal and Golden Delicious) also served as one of the reason for increase of inoculum under favourable conditions. However, the overall results of this study indicated that the fungicide applications against apple scab can be omitted at the beginning of the season and could be a good strategy for saving cost in integrated orchards, if PAD values are lower than 600 ascospores/m^2. Smaller findings were also obtained by Holb and Heijne (2002) and MacHardy *et al*. (1993).

As several factors are involved in calculating the pattern of distribution of ascospores, such as amount of overwintering inoculum, cultivars, size of tree, spacing, wind velocity, leaf fall, leaf wetness, temperature and rate of leaf decomposition results of the present study may not be applicable to all orchards of Uttarakhand Himalayas. Holb *et al*. (2004) showed that if autumn scab incidence, PAD and aerial ascospore concentration are high, there is also a risk of overwintered conidial infection from buds in early spring, which should be taken into consideration if orchard sanitation is used. The potential ascospore dose has potential for numerous uses in scab management (Gadoury and MacHardy, 1986;

MacHardy *et al*., 2001; Li and Xu, 2002) but lack of accuracy or uncertainty in predicting PAD has limited its use in practice (MacHardy, 1996).

Estimates of PAD are useful, when comparing management strategies or control treatments in several orchards. The present investigation reveals the direct influence of overwintering leaves (leaf litter) on the quantum of primary inoculums (PAD) in spring, where other components like LD, PD and cultivars had little effect. It is, therefore, advantageous to adopt measures like 2% urea spray on apple trees and orchard floor at the time of leaf fall with or without the adoption of sanitary measures for enhancing the leaf decomposition and reducing the overwintering inoculums to over 90 through the suppression of pseudothecial development. Such practice reduces the fungicidal application to the minimal necessary to maintain the pathogen population and disease development below an economically damaging level. However, the results clearly indicated that the PAD was not uniformly distributed in farmer's apple orchard or other management practices were applicable in one orchard but not in another orchard subjected to the same weather conditions. Thus more reliable data would be obtained from managed orchard whether autumn treatment of urea with sanitation practices can be used effectively to lower the PAD and, as a consequence, lower the fungicide dose. Therefore, reduction of primary inoculum sources could play very important role in the improvement of effective scab management of apple in Uttarakhand Himalayas.

CONCLUSION

Our results revealed the importance of apple scab disease in the Indian Himalayas. The prevalence of scab was upto 100% in Gangotri valley. It occurred in epidemic proportion in 1996 and again in 2008 in the Gangotri valley (Uttarakashi) of Uttarakhand hills. Control of disease, which essentially relies upon repeated application of fungicides (10-12 spray), does not always end up as an acceptable and profitable venture, especially at the end of small and marginal growers in the region. Besides, excessive use of pesticides continues to be a threat to the highly fragile Himalayan ecosystem. PAD assessments are not time consuming for a trained person but the method could be made simpler to be followed by the grower. It has great impact on disease development in the following season. It varied between 645-387782 ascospores/m^2 orchard floors and the forecast minimum delay of the epidemics (t) based upon PAD ranged from 6-28 days after pink bud stage. PAD was dependent upon plant protection measures undertaken during previous years and the number of chemical applications will vary with the frequency of PAD. Good scab management could be obtained by giving no more than 3-4 fungicide applications and 2% urea spray at the time of leaf fall, if it was backed by estimation of potential ascospore dose during early stage of growing season, even though numerous infection periods were observed during post infection period.

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