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Evidence for loss of ontogenetic resistance of apple leaves against *Venturia inaequalis*

A. Kollar
Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Fruit Crops, P.O. Box 1264, 69216 Dossenheim, Germany

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Abstract

The susceptibility of apple leaves to *Venturia inaequalis* was investigated by assessing disease on individual leaves of seedlings and labelled shoots of orchard trees. Four sets (A–D) of potted seedlings of cv. 'Golden Delicious', which had been grown in a glasshouse, each with approximately 30 mature leaves, were exposed to a high-inoculum orchard. Sets A and B were exposed after each other for 47 and 42 days, respectively. As a result of the six and three infection periods during exposure, 94% and 81%, respectively of the seedling shoot tips in set A and set B became infected. However, due to ontogenetic resistance, disease incidence was low in both sets on leaves which were fully expanded at the beginning of exposure. Set C was exposed during both periods (89 days) in which sets A and B were exposed. Not only were all the seedling shoot tips in set C infected, but also – due to the loss of ontogenetic resistance – nearly all of the mature leaves. Ontogenetic resistance was also lost in set D, which was exposed for 57 days at the end of the growing season. The symptoms on fully expanded leaves on plants in sets C and D were typical and similar to those on young leaves. A time course symptom assessment was performed on leaves which had developed early in the season on labelled, field-grown shoots of cv. 'Golden Delicious' trees. A steady increase of disease incidence was detected, which could not only have resulted from infections followed by extended incubation periods. In addition to the increase of typical lesions on both leaf surfaces, there was also an abundance of diffuse mycelial development on the lower surfaces of the leaves of the field-grown trees.

Introduction

The environmental and the pathogen parameters involved in the epidemiology of apple scab caused by *Venturia inaequalis* (Cke.) Wint., have been described extensively (Anagnostakis and Aylor 1991; MacHardy and Gadoury 1989; Moore 1964, Schwabe 1980). This research focused mainly on fungal inoculum and leaf or fruit wetness requirements for infection. Ontogenetic resistance is considered a dogma and is regarded as an important component for improving scab control strategies. The susceptibility of leaves (Anagnostakis and Aylor 1991; Gessler and Stumm 1984; Kirkham and Hignett 1973; Moore 1964; Schwabe 1979) and fruit (Schwabe 1982; Schwabe et al., 1984; Tomerlin and Jones 1983) decreases with age and maturity of the plant sites. Fully expanded leaves of cv. 'Golden Delicious' or apple rootstock MM 109 have shown almost complete resistance (Gessler and Stumm 1984; Kirkham and Hignett 1973; Schwabe 1979). Keitt and Jones (1926) found that resistance developed more rapidly and to a greater degree on the ventral surfaces as opposed to the dorsal surfaces of the leaves. The degree of resistance on the dorsal surface also depended on the cultivar tested. Furthermore, symptoms characterized as sparse, diffuse and inconspicious developed after a prolonged incubation period of about one to two months. Acquired ontogenetic resistance is considered to be a constant feature in plant development, which, once established, cannot be changed during the course of the further plant development and state of physiology. An increase in scab symptoms late in the season is mainly ascribed to the length of the incubation period,

which is considered to be the only reason why scab symptoms emerge on old, mature leaves.

Recently, Kohl and Kollar (1994) reported that symptoms developed on leaves, which had been healthy for about two months, within an incubation period of less than one month. Although the loss of ontogenetic resistance was most probable when all data were considered, the role of possible extremes of incubation periods could not be completely excluded. These studies were conducted in an orchard to identify the stability or variability of ontogenetic resistance and to show the effects on the susceptibility of the host plant.

Materials and methods

Plant material and orchard site

Orchard trees of cv. 'Golden Delicious' on 'Bittenfelder' rootstocks were planted in 1979. A total of 55 shoots from 23 trees were randomly selected and used throughout the study. Growth of all shoots was marked by tying a thread above the final fully expanded leaf on 4, 11 and 26 May, 7 and 24 June, and 16 July, 1993. Also, 1-to 2-year-old 'Golden Delicious' seedlings approximately 1 m tall with approximately 30 fully expanded leaves were used for the exposure in the orchard. Seedlings were grown in the glasshouse in 15 cm-diameter pots and pruned 3 to 4 cm above ground when shoots had developed more than 35 fully expanded leaves. They were maintained at a temperature of 15–30 °C and a relative humidity of 40–90% and were fertilized as required to ensure continuous and vigorous growth. One week before exposure, the potted plants were acclimated to UV-light in a glasshouse open on one side. At the beginning of exposure, the final fully expanded leaf (leaf 0) of each plant was labelled by cutting off the leaf tip. Four sets of seedlings (A, 36 trees; B, 36 trees; C, 88 trees; D, 151 trees) were exposed as indicated in Figure 1. All the seedlings were comparable (number of leaves, age of respective leaves) and were kept under identical conditions before exposure. Seedlings were watered regularly in the orchard, to ensure further growth. After exposure, the seedlings were returned to the glasshouse and maintained under the same conditions described previously, without additional leaf wetting periods.

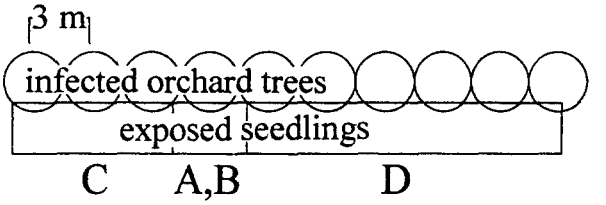

Figure 1. Exposure of 'Golden Delicious' seedlings in the orchard. Approximately 30 mature leaves were present on single shoots of plants. Set A, B (36 trees), C (88 trees), and D (151 trees) were placed near a heavily infected row of orchard trees of cv. 'Golden Delicious' in 1993. Set A was exposed from June 23 to August 9, set B from August 9 to September 20, set C from June 23 to September 21 and set D from September 1 to October 27.

Infection periods and disease assessment

Infection periods were monitored with two electronic orchard environment monitors (KMS-P, Austria and HP 100 Lufft, Germany). Disease development was assessed on labelled shoots of orchard trees on 28 June, 11 August and 8 October. The symptoms on these leaves were recorded as either typical for both surfaces or as diffuse, when sparse or confluent mycelial development without clear demarcation occurred on the lower surfaces of leaves. The position of each leaf on the shoots and leaf scars, which resulted from leaves lost due to hail, wind, insects etc., were recorded. Sets of seedlings were assessed for disease symptoms in the same manner. Symptoms of each set were recorded at the end of the exposure time in the orchard. The second assessment followed incubation in the glasshouse one month later for sets B, C and D. Set A was assessed a second time on 20 September, after the first assessment of sets B and C had been completed.

Results

Disease development on seedlings

The exposure times of the seedlings and the 17 infection periods, recorded according to the criteria of Mills (1944) for light, moderate or severe infection, are presented in Figure 2. Leaves which developed during the time of exposure above the labelled position showed a high disease incidence. In sets A, B, C and D, 94, 81, 100 and 83% respectively, of seedlings were infected above this position. Disease assessment of all single leaves below the labelled position are presented in Figure 3. In sets A and B, only 17% and 10%, respectively, of the first 10 leaves below the labelled position developed symptoms. The percentage of infected leaves for positions 11 to 20 were only 3.0% and 0.8%

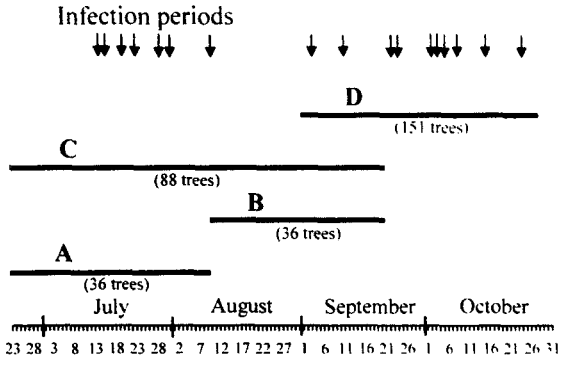

Figure 2. Infection periods and time intervals of exposure of 'Golden Delicious' seedlings (sets A, B, C, D) in 1993. Infection periods (arrows) were determined with electronic scab warning systems by recording leaf wetness requirements for a light, moderate or severe infection according to Mills (1944). Light infection was recorded for period 13, moderate infections for periods 6, 7, 11, 14–16 and severe infections for periods 1–5, 8–10, 12, 17, respectively.

for sets A and B, respectively. The oldest leaves below position 20 were only infected in a few cases in set A. Sixty-one percent of the seedlings in set A and 69% of the seedlings in set B showed no infection at all below the labelled leaves. Considerably more infections were observed on the leaves of sets C and D. In sets C and D, 86% and 63% of the first 10 leaves below the labelled position showed symptoms. At positions 11 to 20 and 21 to 30, disease incidence for set C was 82% and 66% and for set D 32% and 29%, respectively. Only 1.1% of the seedlings in set C and 11% in set D showed no symptoms below the labelled position. Almost all symptoms which occurred on leaves below the labelled position in sets C and D could be characterized as typical lesions, with abundant sporulation on the upper leaf surfaces.

The second disease assessment of sets B, C and D showed that the additional incubation of one month in the glasshouse under similar conditions, did not result in a significant increase of disease incidence. The loss of old leaves, as indicated by the slope of the graphs max. potential disease (Figure 3), was most evident in set C and seemed to be correlated with the duration of exposure in the orchard or the date of disease assessment. Defoliation was due to weather conditions, insect damage and to senescence. Some defoliation also occurred as a consequence of the severe apple scab infection.

Time course of the disease development on orchard trees

Symptoms appeared on all leaves of labelled shoots after an incubation period of about two weeks with the exception of the first basal leaves, which were fully expanded on May 4. The time course of disease development on these leaves from the first labelled interval of the shoots is presented in Figure 4. The first assessment was carried out on June 28, which corresponded to a maximum theoretical incubation period of about 2–2.5 months. Accordingly, subsequent potential incubation periods were about 4 months and 5 to 6 months (Figure 4). On the first assessment date, 35% of leaves were infected. This increased to 77% and 94% on the following assessment dates. On the first assessment date, 25% and 18% of all leaves showed typical symptoms on the upper and lower surfaces, respectively. On the upper leaf surfaces, there was an increase to 41% and finally to 65%, and on the lower surfaces a decrease to 6.7% followed by an increase to 63%. Diffuse symptoms on the lower surfaces were detected on only 1.1% of leaves on the first date of disease assessment which increased to 71% and 84% of the leaves on the following dates.

Discussion

In this study, it was demonstrated that ontogenetic resistance of apple to *V. inaequalis* is variable and is an effect of orchard environmental factors. This supports the statement made by Hignett (in: Kirkham and Hignett, 1973): "This question of mature resistance is something which has a convenient label but there seems to be fog of ignorance." Recently, Kohl and Kollar (1994) reported a loss of ontogenetic resistance on leaves of shoots of orchard trees. In contrast to the present study, labelled shoots were not used for disease assessment, hence the interpretation of the results was more difficult. The use of labelled individual shoots for the disease assessments allowed a precise time course of disease development to be made. This is not attainable with randomly selected shoots, due to their variable growth. Furthermore the use of labelled shoots allowed a continuous evaluation of symptom development on each individual leaf. Diffuse symptoms were detected only on the lower surface of the leaves of shoots in the orchard, which occurred, as described by Sutton et al. (1976) in the late summer. Assuming that only the duration of incubation responsable for these

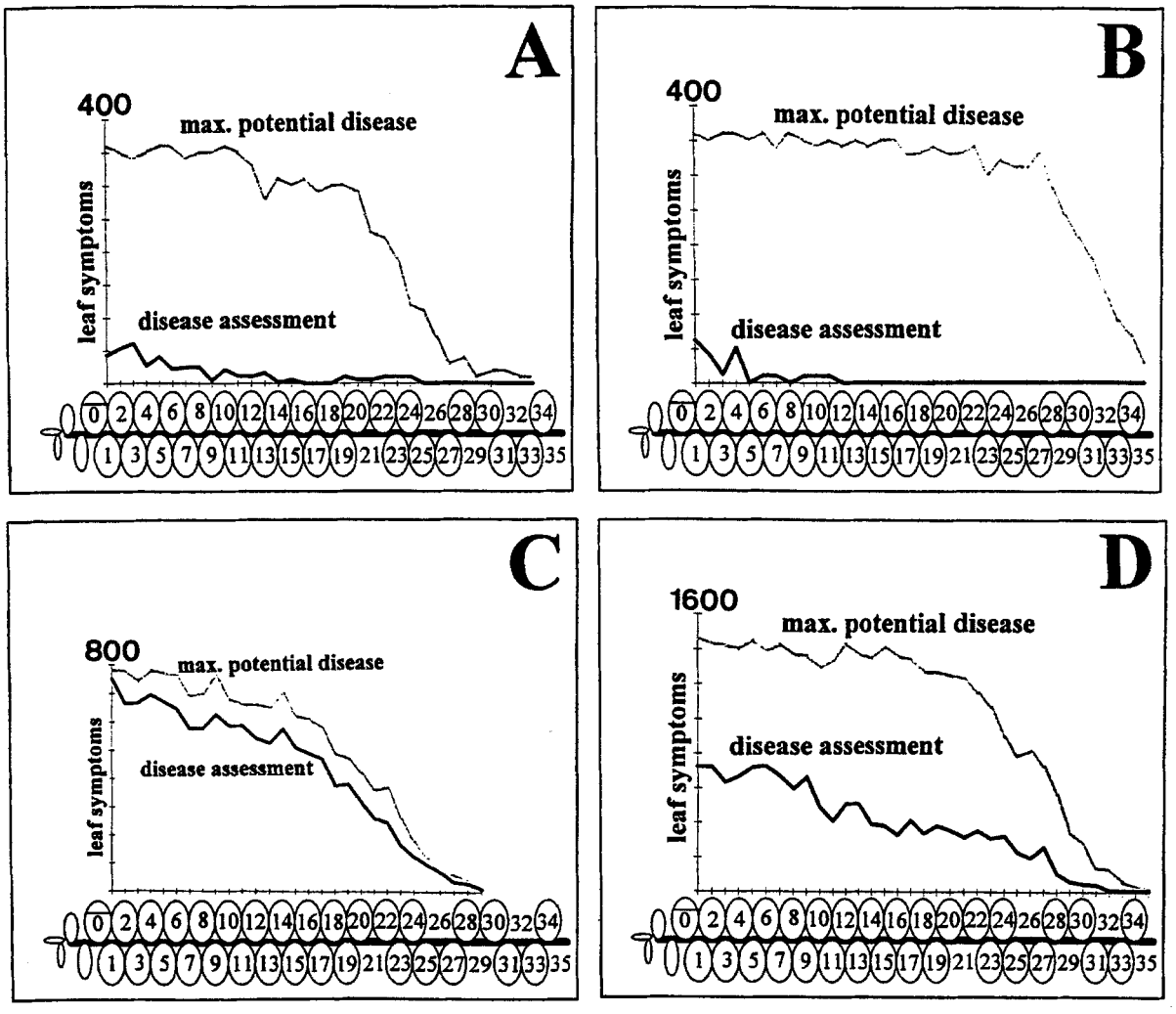

Figure 3. Disease assessment of sets of 'Golden Delicious' seedlings, considering data of all the single leaves in 1993. At the beginning of exposure in the orchard (time intervals and orchard set up see Figures 1 and 2), the final fully expanded leaf of each of the seedlings were cut at the tip and designated as leaf 0. Disease assessment began with leaf 1. The predominant symptom of each leaf was recorded irrespective of the number of lesions on the same leaf. Numerical values for each leaf were 1, 0.5, 0.1 for lesions with abundant, low sporulation, or for barely visible symptoms, respectively. Values for leaf symptoms (y-axis) are the summarized values of all leaves for each leaf position (x-axis). max. potential disease: theoretical graph assuming that all leaves still attached at the date of assessment would carry lesions with abundant sporulation. A: set A; B: set B; C: set C; D: set D. Sets B, C, and D were assessed at their end of exposure period. Set A was assessed together with sets B and C.

late symptoms, a period of about 2.5 months would have been necessary to initiate substantial mycelial growth and a further three months to develop strong visible symptoms. The steady increase in typical symptoms on the upper surfaces of the leaves more clearly demonstrated the reduction of ontogenetic resistance. Here, new symptoms appeared continuously within 5 to 6 months which cannot be a result of the extended incubation periods of the three or four infection periods, which occurred early in the season. Moreover, resistance is thought to be acquired faster and more complete on the upper surface of leaves. The increase of typical symptoms on the last assessment date were nearly identical on both the lower and upper leaf surfaces. The apparent decrease in disease on the lower surfaces at the second date of assessment was caused by interjacent (not clear diffuse or typical) or variable symptoms.

The results with potted plants clearly indicated that nearly a complete loss of ontogenetic resistance is pos-

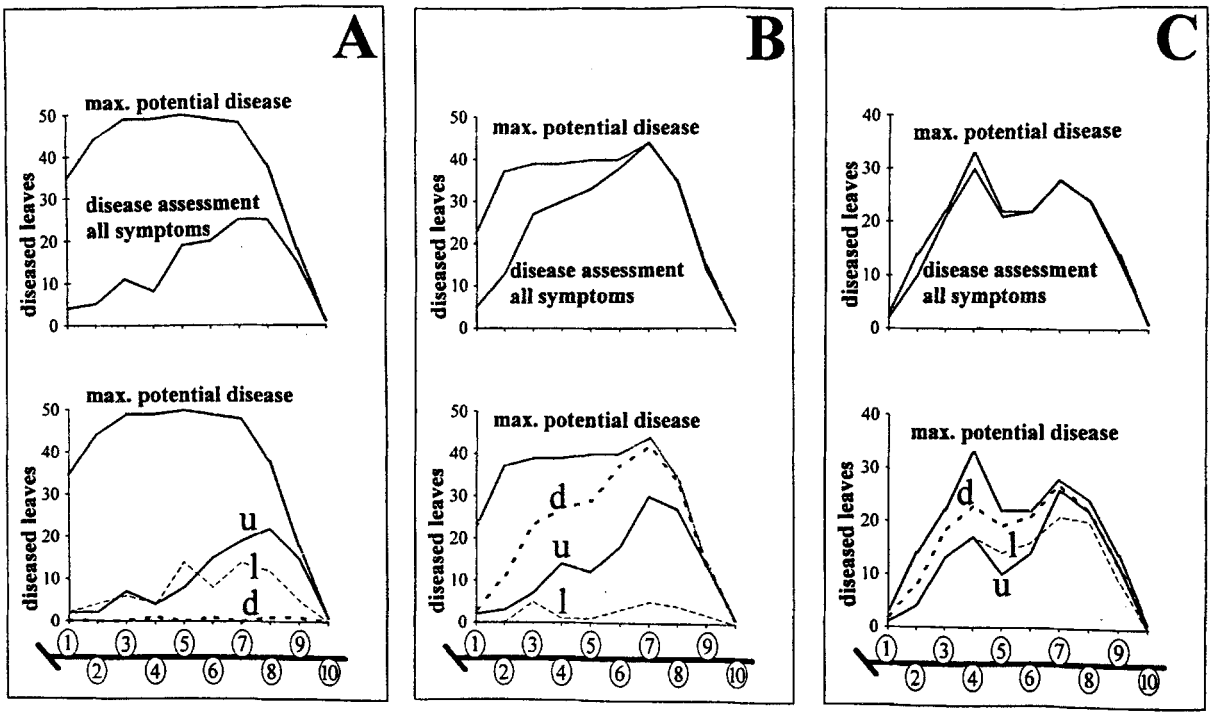

Figure 4. Time course of disease assessment of individual shoots of orchard trees cv. 'Golden Delicious' in 1993. All of the 55 shoots were evaluated, starting with the first basal leaf of the shoot up to the youngest fully expanded leaf as recorded on 4 May. Dates for disease assessment were A: 28 June, B: 11 August, C: 1 October. The numerical data used for graphs were 1 or 0 corresponding to the presence or absence of leaf symptoms (y-axis: number of diseased leaves). max. potential disease: theoretical graph assuming that all leaves still attached at the date of assessment show a symptom. u: typical lesions formed on the upper surface and l: on the lower surface of leaves. d: diffuse mycelial development on the lower surfaces of leaves. Symptom expression was assessed according to the predominant appearance.

sible on all leaves, including the oldest. These experiments have proved that mature leaves of different ages can become infected when orchard environmental factors effect ontogenetic resistance. Furthermore, the role of extended incubation periods, often assumed to be the only reason for the late appearance of symptoms, was disproven. This was possible because of the accurate knowledge of plant features and the defined time intervals of exposure and incubation periods. Seedlings exposed in the orchard for approximately 1.5 months showed very low disease incidences on mature leaves, which were comparable to those commonly observed after an artificial infection and long incubation periods in the glasshouse. An extention of the exposure period to 2 and 3 months resulted in the loss of ontogenetic resistance. The symptoms which developed could be characterized mostly as class 4 symptoms (Chevalier et al., 1991) with abundant sporulation; there was no longer any visible effect of plant resistance.

Reasons for the loss of resistance may be related to either the fungus, the plant, or both. An effect on the old leaves by infected young leaves from the tip of the plants or the production of especially adapted conidia can be excluded, since the shoot tips of all plants showed a high disease incidence. The frequency of infection periods had no accumulative effect. Set A was exposed for about 1.5 months with six infection periods and set B for the second 1.5-month interval with only three infection periods. Plants in set C, which were exposed to both exposure times lost their resistance almost completely. This implies that the infection to set C occurred during the last three infection periods, which occurred during the second 1.5-month interval of exposure. Moreover, it is probable that the last two infection periods were responsible for the infection, because the first occurred very close to the exposure period of set A. Set D was exposed for about two months with 10 infection periods but loss of resistance was not as strong as that observed in set C. Considering all the data, it seems probable that the exposure time in the orchard was directly related to the loss of ontogenetic resistance. This effect was evident

after an exposure period of at least two months and increased to a nearly total loss of resistance after about three months. The reasons for the change in resistance reaction could be environmental factors in the orchard, which may alter the physiology of the plants during the period of exposure.

The importance of these findings might also be correlated with symptoms which emerge late in the season on old leaves. These lesions were thought to originate from early infections, followed by a long incubation period. The curative action of fungicides is restricted to a few days only. Thus, these late symptoms cannot be considered in control strategies using fungicides. Because new infections can occur on all leaves, regardless of their degree of maturity or age, there could be a significant increase of diseased leaves late in the season and thus in an enhanced level of ascospores in the following year. Future investigations should improve the knowledge of how environmental factors lead to the loss of resistance and of factors *in planta* responsible for this reversible ontogenetic resistance.

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