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Comparative studies on the components of innate partial resistance to Venturia inaequalis (Cke) Wint in nine apple cultivars

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COMPARATIVE STUDIES ON THE COMPONENTS OF INNATE PARTIAL RESISTANCE TO *VENTURIA INAEQUALIS* (CKE.) WINT. IN NINE APPLE CULTIVARS

BY

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DISSERTATION

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DEDICATED

TO

MOM

Thank you, for your unending love, faith, and support.

Hold fast to dreams
for if dreams die,
life is a broken
winged bird that
cannot fly.

— Langston Hughes

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ABSTRACT

COMPARATIVE STUDIES ON THE COMPONENTS OF INNATE PARTIAL RESISTANCE TO *VENTURIA INAEQUALIS* (CKE.) WINT. IN NINE APPLE CULTIVARS

by

Cheryl A. Smith
University of New Hampshire, September, 1992

The components of partial resistance to *Venturia inaequalis* and the expression of resistance were quantified in nine apple cultivars ('Delicious,' 'McIntosh,' 'Stayman,' 'Mustu,' 'Paula Red,' 'Ida Red,' 'Golden Delicious,' 'Spartan,' and 'Rome') under field conditions. Ascospore productivity was influenced by cultivar, and was greatest on 'Rome' (476 spores/cm^2 leaf), but cultivar did not affect the rate of ascospore maturation significantly.

Relative conidium productivity/lesion was influenced by cultivar, and ranged from 30,126 conidia/lesion on 'Golden Delicious' leaves to 4,443 conidia/lesion on 'Delicious' leaves. The infectious period (length of time conidia are produced) was longest on 'Golden Delicious' (94 and 68 days) and shortest on 'McIntosh' (51 and 52 days). An early buildup of scab occurred on cultivars with lesions that had high conidium productivity during the first 10 days after lesion appearance. A non-destructive technique that allowed repeated removal of conidia from scab lesions is described.

Incubation period was not influenced by cultivar, but it was correlated with leaf age: the greater the age of a leaf when infected, the longer the incubation period. An ontogenic resistance mechanism was apparently operational in all cultivars by the 12th day after a leaf unfurled.

The area of exposed tissue at each fruit bud growth stage measured and the area of the youngest five leaves on a shoot differed among the cultivars. The rate of extension shoot

growth (leaves emerged/week) did not differ among the cultivars. Each year, phenological development of the fruit buds differed among the cultivars on each observation date except the final observation at fruit-set.

The cultivars are ranked for resistance, based on incidence and severity of foliar scab as follows: least resistant are 'McIntosh' and 'Rome,' moderately resistant (disease reduced by 41-76 %, relative to 'McIntosh') are 'Stayman,' 'Mutsu,' 'Spartan,' 'Golden Delicious,' and 'Delicious,' and highly resistant (disease reduced 92 %, relative to 'McIntosh') are 'Ida Red' and 'Paula Red.'

INTRODUCTION

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is the most important disease of apples in the northeastern United States. Near-complete control of the fungus is required to maintain fruit quality sufficient to meet fresh market demands for "perfect fruit." The development of apple scab under favorable disease conditions is determined by the inoculum level (primary and secondary inoculum) and the innate resistance of the cultivar infected. Resistance is defined as the ability of a host to hinder the growth and/or development of a pathogen (103), and partial resistance is a form of resistance which is expressed quantitatively as a reduction in the size and number of sporulating lesions (46) Susceptible apple cultivars do vary in resistance to apple scab, but there is practically no information on quantitative differences in resistance and relatively few studies have compared cultivars for the expression of partial resistance to *V. inaequalis*. Aldwinkle (5) surveyed research and extension workers in 1984 for their ratings of susceptibility to scab for each cultivar based on field observations. In the northeast, 'McIntosh' was rated most susceptible, while 'Delicious' was moderately resistant. A study in France (96) that compared 24 cultivars, grouped the cultivars into three categories: least susceptible, moderately susceptible, and very susceptible, based on their relative scab resistance. Quantitative and qualitative differences in infection that distinguished the three categories were not stated, however.

There are several components of a pathosystem that may potentially contribute to partial resistance (101, 95, 127). With apple scab, these components include the length of the incubation period (the period between inoculation and the first appearance of a sporulating lesion), lesion size, the number of conidia produced on a scab lesion, the

number of pseudothecia per scab lesion, and the number of asci per pseudothecium. Cultivar traits may contribute to avoidance (i.e. escape). These traits are not expressions of genetic resistance, but they may affect inoculum level: the time/rate of leaf-fall, the rate of leaf litter decomposition, the amount of exposed susceptible tissue at different host phenological stages, and the time and rate at which the phenological stages occur in relation to the rate of ascospore maturation.

Several studies have provided a limited amount of information about the components of partial resistance to apple scab. A study that compared pseudothecial density and ascal density on 'Delicious' and 'McIntosh' leaves infected with *V. inaequalis* indicated there was no significant cultivar effect (42). Szkolnik (120) compared thirty cultivars for their relative susceptibility to foliar infection in a greenhouse study and reported that 'Golden Delicious' had 15% fewer lesions than 'McIntosh,' and that 62% fewer conidia were produced on the lesions. Two studies (62, 87) reported that resistance to scab may be associated with a cultivar's effect on ascospore production. In the study by Jeger et al. (62) ascospore productivity was reduced 88% on 'Golden Delicious' and 81% on 'Red Delicious' compared to 'McIntosh.' A study by Moller (87) reported the peak number of ascospores discharged from infected 'Golden Delicious' leaves was reduced 75% and 65% compared with infected leaves on 'Winesap' and 'Granny Smith,' respectively. The two studies also indicated that resistance may be due, in part, to the rate ascospores mature and the timing of tree growth stages. The peak period of ascospore productivity was not coincident with the peak production of susceptible tissue in the Jeger et al. study (62), and Moller (86) reported no differences in the rate of ascospore maturation among six apple cultivars. Gessler and Stumm (48) and Schwabe (107) reported that apple leaves become more resistant to infection by *V. inaequalis* as they expand and mature, a phenomenon which is referred to as ontogenic resistance, a resistance inherent in all apple cultivars (46). It is unknown, however, if the interval between leaf emergence and the time leaves reach full ontogenic resistance is similar for all apple cultivars. These studies have provided

some information on the components of partial resistance, but there are no field data that quantify and compare several components of innate partial resistance to apple scab among commercial apple cultivars.

The objectives of this dissertation were:

1. to quantify the partial resistance to *Venturia inaequalis* in nine commercial apple cultivars (as compared to the susceptible cultivar 'McIntosh').
2. to quantify the major components of innate partial resistance and determine the importance of those components in scab buildup.

CHAPTER I

REVIEW OF THE LITERATURE

The History of the Apple and Certain Apple Cultivars

The modern apple is believed to have originated in several gene centers: south-eastern Asia, China, Japan, the Mediterranean, Europe, south-central Russia, or the Balkans (20, 19). The first attempts of apple cultivation may have been in the Middle East or south-eastern Europe, with the eventual spread of those techniques by the Greeks and Romans. Richard Harris, fruit expert to Henry VIII (~1500 AD), imported many 'new' apple varieties to Britain from Europe. These imported varieties were planted in an orchard in Teynham, Kent (20). These importations were part of the early efforts made toward the improvement of apples in England. At the end of the eighteenth century, the demise of 'Golden Pippin' trees, caused by *Nectria galligena* canker, prompted Thomas Knight to begin making deliberate crosses of known apple cultivars. His work along with the Royal Horticultural Society stimulated interest in breeding new apple cultivars and resulted in the establishment of fruit research stations such as those at East Malling in Kent, England. The apple was imported to North America in the early seventeenth century. Early propagation was by seed, but exceptional cultivars were eventually grafted onto seedling rootstock and active breeding programs were begun (20).

The cultivar 'McIntosh' was a chance seedling discovered by John McIntosh around 1811 in Dundela, Ontario, Canada (20, 123). He began growing and selling seedlings of inconsistent quality in 1820. By 1835, grafted 'McIntosh' trees were being sold from the orchard in Dundela. The apple was originally called 'Granny's Apple.' In 1836 the apple was renamed 'McIntosh Red' and eventually 'McIntosh' when it was introduced to the public and promoted by Allen McIntosh in 1870. The first printed reference to 'McIntosh' was in Fruits and Fruit Trees of America by Downing in 1876. It was not until 1900,

however, that 'McIntosh' became widely known (123). The marked susceptibility of 'McIntosh' to apple scab was probably why its acceptance was slow.

The parentage of 'McIntosh' is speculative. Strong evidence, based on growth characteristics and planting distributions, indicates that 'Fameuse' and 'Alexander' (an old Russian cultivar), may have been the parents (123). Three management practices have helped to improve the commercial value of 'McIntosh': (1) fungicides for control of scab, (2) the use of hormones to reduce pre-harvest drop, and (3) controlled atmosphere (CA) storage. These developments, along with high yields and good fruit quality, have established 'McIntosh' as one of the most important apple cultivars in North America, particularly in the northeast.

The cultivar 'Delicious' or 'Red Delicious' was discovered as a chance seedling by Jesse Hiatt in his orchard in Peru, Iowa around 1870 (20, 81). Haitt called the apple 'Hawkeye,' and entered it in a fruit show in Louisiana, MO sponsored by Stark Nurseries, in 1893 and 1894 (81). After the 1894 Fruit Show, Stark Nurseries purchased the propagating rights and renamed the apple 'Delicious.' The 'Yellow Bellflower' is believed to be one of the parent trees of 'Delicious' because it has the five-crowned fruit that is characteristic of 'Delicious' and the 'Delicious' seedling was found growing near it. 'Starkrimson' is a widely grown spur-type sport discovered on a 'Starking Delicious' tree in Hood River, OR in 1951 (81). The fruit was uniform and attained full color one month earlier than usual. Stark Nurseries paid $25,000 for the tree, the highest amount paid for one tree up to that time. No cultivar of American origin has ever gained acceptance as quickly as 'Delicious,' and it is now the world's most popular variety. In 1922 a 6,000 pound stone monument was placed in Winterset, Iowa to commemorate the original 'Delicious' apple tree.

The cultivar 'Golden Delicious' was discovered by Anderson H. Mullins as a chance seedling from an open-pollinated 'Grimes Golden' orchard in West Virginia in 1890 (20, 82). The apple was originally called 'Mullins Yellow Seedling.' The exceptional keeping

quality, size, color, and taste of the fruit convinced Mullins to send three apples to Stark Nurseries who later that year purchased the propagating rights for the tree for $5,000 (82). In 1914 Stark Nurseries renamed the cultivar 'Golden Delicious' believing that the association with the name 'Delicious' would help speed the acceptance of the golden apple. It is now one of the two or three most popular apples in the world.

The cultivar 'Rome' originated in Proctorville, Ohio in 1817 (20, 89). It was discovered by Joe Gillet as a sprout from the rootstock of an unplanted tree he had purchased from Putnam's Nursery in Marietta, Ohio in 1816 (89). The quality of the fruit was so exceptional that the tree was being propagated for commercial sale in 1928. George Walton renamed the apple 'Rome Beauty' in 1936. It was introduced in 1948 by H. N. Gillet at the Ohio Convention of Fruit Growers.

The cultivar 'Ida Red' was bred by Leif Verner at the Idaho Experiment Station, Moscow, Idaho (18, 20). It was selected in 1935 from the progeny of a cross between 'Jonathan' and 'Wagener.' It was introduced in 1942.

The cultivar 'Paula Red' was discovered as chance seedling of unknown parentage in 1960 by Lewis Arends in Sparta, MI (18). Hilltop Orchards and Nurseries introduced the cultivar in 1967.

The cultivar 'Spartan' originated in Summerland, British Columbia, Canada (20)from a cross between 'McIntosh' and 'Yellow Newton Pippin' made in 1926 at the Dominion Experiment Station. The tree first fruited in 1932, and was introduced to the public in 1936.

The cultivar 'Mutsu' (also called 'Crispin') originated in Kuroishi, Aomori Prefecture, Japan (18). It was selected from a cross in 1930 between 'Golden Delicious' and 'Indo,' named by the Japanese Horticultural Association in 1948 and introduced to the United States. The cultivar was patented in Japan in 1949.

Genetics of the Resistance in Malus

The apple belongs to the Rosaceae (rose) family (19). Apples and other pome fruits are in the subfamily Pomoideae. Plants in this family have fruits with 2-5 carpels enclosed in a fleshy covering. The genus *Malus* has approximately 20-30 species and numerous sub-species that are small fruited (crab apples). Most *Malus sp.* are self-incompatible but cross freely (136). This self-incompatibility makes it nearly impossible to obtain inbred parental lines which are homozygous for various factors.

Many early workers noted distinct differences in the susceptibility of apple cultivars to *Venturia inaequalis* (3, 68, 63). Although numerous cultivars exhibited reduced levels of scab infection, none was completely immune. In the early 1930's, Rudloff and Schmidt (106) noted that several crab apple species appeared resistant to scab infections. They also noted that the cultivar 'Antonovka' possessed a high level of resistance, but an understanding of the basis of this resistance was necessary before successful breeding for apple scab resistance could be realized.

Two types of resistance to *V. inaequalis* exist in *Malus sp.*: qualitative or monogenic resistance, determined by a single major gene, and quantitative or polygenic resistance determined by multiple minor genes (19, 46, 91, 136). Monogenic resistance is expressed qualitatively by differences in symptoms such as pin-point lesions, chlorotic flecks, or lack of visible symptoms. Polygenic resistance, frequently referred to as partial resistance, is often expressed quantitatively as a reduction in the number or size of sporulating lesions (46, 91, 136). The expression of polygenic resistance is sensitive to the environment while the expression of monogenic resistance is not .

The crossing of susceptible and resistant parents can help determine if the resistance is controlled by a single major gene, provided that the susceptible parent is homozygous recessive for the major gene. A segregation ratio of 1:1 (resistant:susceptible) in the F_1 progeny, or 3:1 in the S_1 progeny indicate the resistance is controlled by a single major

gene (19, 58). Segregation ratios other than 1:1, such as 3:1 or 7:1 in the F_1 generation of a cross between susceptible and resistant parents, and 15:1 or 63:1 in the S_1 progeny indicate polygenic resistance (111).

It is generally accepted that monogenic resistance is less stable than polygenic resistance (19, 46, 47, 91). With monogenic resistance, a single mutation in the pathogen may overcome the single resistance gene and result in a new race of the fungus (34, 46, 126). With polygenic resistance, several matching mutations by the pathogen are required, and the likelihood of this occurring is inversely proportional to the number of minor genes for resistance present in the host. Crop breeders dislike breeding for polygenic resistance because (i) the minor genes are often lost in backcrossing, (ii) the frequency of resistant individuals can't be predicted, and (iii) the continuous variation in the progeny makes selection a formidable task because it is difficult to identify slight reductions in susceptibility (47, 91).

Monogenic resistance

The existence of a major gene that conferred resistance to *V. inaequalis* was discovered by Hough in 1944 (57). He observed near immunity in progeny from a cross between 'Rome Beauty' x *Malus floribunda* 821 made by Crandall (23). The seedlings from a sib-cross segregated 1:1 and were either highly resistant or susceptible, indicating that a single dominant gene was responsible for the resistance (57). This gene was named V_f for *Venturia inaequalis* and *M. floribunda.*

A formal breeding program for the development of scab resistant cultivars was begun in 1945 by Shay and Hough at Purdue University and the University of Illinois, respectively (136, 135). In 1948, Rutgers University was included in the program and the program then became known as the PRI Cooperative (131). A classification scheme based on foliar symptoms was developed to rate the resistance of the progeny from the controlled crosses: 0 = no macroscopic symptoms, 1 = pin point pits, no sporulation, 2 = chlorotic or necrotic lesions, no sporulation, M = a mixture of necrotic, nonsporulating, and sparsely

sporulating lesions, 3 = restricted necrotic lesions with sparse sporulation, 4 = extensive, abundantly sporulating lesions (58, 113). Class 4 was considered susceptible. Classes 1, 2, M, and 3 were considered resistant, although there has been some debate over the inclusion of class 3 as a resistant reaction (47, 74).

The breeding program identified several *Malus* species that were heterozygous for a single dominant gene for resistance. It became necessary to determine if one gene, several allelic (genes at a single locus) genes, or non-allelic (independent) genes from these various *Malus* species determined resistance to scab. When resistant F_1 progeny from a susceptible x resistant cross were backcrossed to a susceptible parent, a 3:1 (resistant:susceptible) ratio in the backcross generation indicated that the genes were non-allelic (29, 134). A total of eleven *Malus* species and selections that carried the V_f gene for resistance were identified by the breeding program (29, 133). A fifth major gene, V_m was identified with the appearance of Race 5 of the fungus(132). Williams and Brown (132) determined that *M. micromalus* and *M. atrosanguinea* 804 possessed two independent resistance genes, one at the V_f locus and another V_m which induced the pit-type reaction. Later, three additional resistance genes were identified: V_r - from *M. pumila* 12740-7A (a 'Russian' selection), V_b - from Hansen's baccata #2, and V_{bj} - from *M. baccata jackii* (29). Another resistance gene, V_a was identified in France in progeny descended from 'Antonovka' (75).

Polygenic Resistance

The existence of polygenic resistance has been noted for many years in apple cultivars. Johnstone (63), for example, commented that several commercial cultivars were relatively resistant to scab, but he did not state how the resistance was expressed. He believed that the resistance was due to some property of the cell contents. Rudloff and Schmidt (106) noted a high degree of resistance in 'Antonovka' compared to the other *M. pumila* varieties. Keitt and Langford (69) considered 'Ben Davis' and 'Yellow Transparent' somewhat resistant based on relative estimates of scab infection. In a survey of *Malus* species and named varieties, Hough (57) noted that the distribution of resistance in progeny from

crosses of cultivated apples was either near normal or skewed, indicating that the resistance was polygenic. It appeared to him that most apple cultivars possessed minor genes for resistance. The differences in the minor genes carried by one cultivar or another were usually expressed quantitatively as differences in the number of scab lesions (47). Polygenic resistance to *V. inaequalis* has been reported to be expressed as a reduction in lesion size, reduced sporulation on lesions, reduced production of ascospores, or an increase in the incubation period (95). Also, when resistant F_1 seedlings were backcrossed to susceptible cultivars, the ratios of resistant to susceptible progeny ranged widely rather than the 1:1 ratio expected if resistance was controlled by a major gene, and this is considered evidence that the resistance was controlled by minor genes (27).

Evidence of polygenic resistance was also obtained for several small-fruited *Malus* species. The resistance of 'Russian' selection R12740-7A (*M. pumila* Mill.) was demonstrated to have at least three qualitative genes and two or more quantitative genes (27, 136). Polygenic resistance has been identified in five other Malus species: *M. baccata* (selected seedlings), *M. sargenti* 843, *M. sieboldii*, *M. torringo*, and *M. zumi colocarpa* (75, 110).

Minor genes may act as modifiers that confer resistance in species that also contain a major resistance gene. Rouselle et al. (1974) noted weak sporulation in some seedlings possessing V_f resistance that were exposed to high inoculum. They postulated that cumulative minor genes increased the resistance controlled by V_f. The level of modifier genes in the progeny could be increased or decreased depending on the number of modifier genes in the backcross parent (73, 105).

It is very difficult to identify the genetic basis of polygenic resistance in commercial apple cultivars. The phenotypic expression of minor genes can be observed by comparing the relative resistance to scab in commercial cultivars. A survey of apple specialists in the United States and Canada, based on their general impressions of resistance or susceptibility to scab, resulted in four relative susceptibility ratings for 51 cultivars for three broad

geographic regions (5). The four rating classes were resistant, slightly susceptible, moderately susceptible, and highly susceptible. The ratings for the various cultivars varied among the geographic regions as well as within a region. Olivier et al. (96) ranked important commercial cultivars into three susceptibility groups: slightly susceptible, moderately susceptible, and highly susceptible. The results of these three studies conflict in the rankings of some of the cultivars. Hough (57) gave two possible explanations to account for the different rankings of cultivar susceptibilities in different studies: (1) the hosts may have been clones of genetically different seedlings of the same species and (2) the pathogen may have differed (the races or strains of the fungus present may have varied for a given location and for the cultivars). All of these studies were subjective, i.e., they were not based on a procedure that allowed quantitative comparisons of the cultivars. There appears to be a need for a standardized method of ranking the relative susceptibility to scab in commercial apple cultivars. The clone of apple should be noted, the geographic area of the study should be limited, and the races of the pathogen present should also be determined and noted. A standardized inoculum containing a mixture of isolates collected from various cultivars would be ideal if used under controlled conditions.

THE PATHOGEN: Classification, Genetics & Inheritance, & Races

Venturia inaequalis (Cooke) Winter is classified as an ascomycete in the order Pleosporales, family Venturiaceae (80). The conidial stage *Spilocea pomi* Fr. was named by Fries in 1819 (35). Cooke described the sexual stage, and in 1866 named the fungus *Sphaerella inaequalis*. Winter transferred the fungus to the genus *Venturia* and renamed it *Venturia inaequalis*.

Venturia as a Genetic Tool

Venturia inaequalis is heterothallic, and has a sexual reproductive phase (72). The eight ascospores within an ascus are derived from a meiotic division followed by one

mitotic division, and this facilitates the study of the line of nuclear descent and inherited characteristics. The fungus is haploid throughout most of its life cycle, and this provided an excellent opportunity for investigating the inheritance of pathogenicity without the complicating effects of variation due to heterokaryosis (31). *Venturia inaequalis* is composed of many biotypes with different morphological and physiological characteristics as well as different pathogenic capabilities (63, 65, 92, 98). The fungus is similar to the obligate biotrophs in that it remains in close association with host tissues for long periods. Unlike the obligate biotrophs, however, *V. inaequalis* is easily cultured and mated *in vitro* and does not possess haustoria (69, 70). Cultures of *V. inaequalis* respond well to mutagenic agents, and they remain stable for many years.

The *Malus* hosts of the pathogen also possess certain advantages for genetic research and pathogenicity studies: (i) they can be hybridized, (ii) the cultivars can be held constant by vegetative propagation, (iii) they possess both monogenic and polygenic resistance (74, 95, 105, 113), and (iv) the leaves usually provide abundant sites for cytological studies on the infection process and disease development.

Venturia inaequalis has excellent adaptations for genetic and physiological studies similar to those of the 8-spored *Neurospora sp.* that have served as models for Ascomycete genetics, but, in addition, *V. inaequalis,* provides the aspect of pathogenicity which *Neurospora* lacks (31, 130). Historically, *V. inaequalis* and its *Malus* hosts have provided one of the best systems for studying the genetics of host-parasite interactions in plants.

The Cytology of Venturia

The development of the ascocarp of *V. inaequalis* was studied first by Frey (35), and later by Keitt and Palmiter (72) and Boone (10). The ascocarp, or pseudothecium, is initiated by a helical growth at the apex of a hypha. The coiled ascogonium (female gametangium) grows in size and eventually becomes surrounded by a wall. A trichogyne is formed which extends through the wall of the ascogonium. The apex of another vegetative hypha of the opposite mating type differentiates into a multinucleate antheridium

(male gametangium). Nuclei in the antheridium pass through the trichogyne into the ascogonium where they pair with nuclei in the ascogonium to form a dikaryon (35, 72). Backus and Keitt (6) observed the formation of ascogenous hyphae from papillae arising from the periphery of the ascogonium. The asci are initiated by crozier formation (6, 72). The penultimate or ascus mother cell of the crozier is binucleate. The two nuclei, each possessing a different compatibility factor, fuse immediately after the penultimate cell is formed. The ascus elongates and the nucleus divides meiotically to form four haploid nuclei which then divide mitotically to form eight haploid nuclei (6, 35). Backus and Keitt (6) noted that the orientation of the spindles was longitudinal during the first division and longitudinal, oblique, or transverse during the second and third divisions. Sister nuclei are arranged in the ascus in pairs in serial order from base to apex, indicating that segregation occurs during the second meiotic division. Backus and Keitt (6) however, found that segregation could take place in either the first or second meiotic divisions.

The spores are uninucleate when first formed, but the nucleus and cell later divide to form the familiar two-celled ascospore. The vegetative phase of *V. inaequalis's* life cycle is initiated when ascospores germinate, infect the host, and form subcuticular stromata and visible lesions. The mycelium is septate and branched. The cells of the mycelium are uninucleate, and the conidia are usually uninucellular and uninucleate (12).

The Genetics of Venturia

Keitt and Palmiter (72), and Keitt and Langford (69) were the first to demonstrate, through crosses with 40 monosporic isolates, that the fungus is heterothallic. They noted that the forty isolates segregated into two groups of twenty with reference to their sterility or sexual compatibility. Both studies determined that *V. inaequalis* is hermaphroditic but self-sterile, intra-group incompatible and inter-group compatible. The results of these studies indicated that the compatibility factor was of the one-locus, two-allele type termed bipolar heterothallism. The two compatibility groups were designated mating types (+) and

(-) (114). These studies laid the groundwork for further studies on the inheritance of pathogenicity and other characters.

Aderhold (2) and Wiltshire (139) described differences in the pathogenic capabilities of different isolates of *V. inaequalis*. Keitt and Jones (68) and Johnstone (63) also noted that some of the cultures caused typical scab lesions on some apple cultivars but not on others. Palmiter (98) observed the variability in cultural characteristics and pathogenic capabilities in 36 monoconidial isolates from fourteen apple cultivars from several geographic locations. He concluded that the fungus was composed of several strains that varied in morphological and physiological characters as well as in pathogenic capabilities. Nusbaum and Keitt (92) also noted variability in the pathogenic capabilities of two *V. inaequalis* isolates on four apple cultivars.

Because it is haploid throughout the parasitic stage, *V. inaequalis* is an ideal organism for studying of single gene pairs and their relationship to parasitism. Keitt and Palmiter (72) were the first to demonstrate segregation of factors for virulence to different commercial cultivars. Each of three cultivars was infected by each of eight monoascosporic isolates that had been isolated from a single ascus. Four of the isolates that infected 'McIntosh' and 'Yellow Transparent' caused typical sporulating lesions, but the other four isolates caused only flecks to form on the leaves, with no conidial production. Keitt and Langford (69) observed segregation of factors for virulence to seven of nine cultivars they tested. In related studies involving color mutants of *V. inaequalis*, the factors for pathogenicity segregated 1:1, and it was hypothesized that a single gene was involved, with multiple alleles controlling the pathogenicity to different apple cultivars.(70, 114).

Mustard gas and ultraviolet light were used to induce morphological, color, and biochemical mutants of *V. inaequalis* which were employed primarily as genetic markers for studies on the inheritance of pathogenicity (12, 15, 66). Most of the morphological mutants differed from normal isolates in growth rate and conidial production. The majority of the color mutants were white, but yellow, green, pale, pink, and brown mutants were

also produced. The color mutants grew at a normal rate, and although their characteristic colors were imparted to the sporulating lesions, the color character was shown to be independent of pathogenicity. Some of the biochemical mutants were nonpathogenic. However, it was possible to restore their pathogenicity by topical applications of the required nutrients to the leaf surface, indicating that the inheritance of pathogenicity was not altered by the mutation. Most of the factors for color mutants and all the morphological and biochemical mutants were inherited as if single-gene controlled.

Keitt et al. (71) reported that pathogenicity to 'Haralson' and 'Wealthy' was determined at one locus and pathogenicity to 'McIntosh' and 'Yellow Transparent' at another locus, with no linkage between the two loci. Boone and Keitt (13) described five additional pathogenicity genes at different loci. Six more genes controlling pathogenicity were identified on apple selections used in the resistance breeding program at Purdue (137), and another six loci were identified on several crabapple cultivars (7). The genes that controlled pathogenicity were given the symbol "p," for pathogenicity, and were numbered consecutively p-1 through p-19 (7, 13, 137). The superscript "+" was used to denote the lesion-conditioning allele, and the absence of the superscript denoted the fleck-conditioning allele. Keitt and Boone (65) determined that the fleck-conditioning alleles were epistatic to the lesion-conditioning alleles for pathogenicity to the same cultivar.

The haploid chromosome number of *V. inaequalis* is seven (25). Linkage of related genes on the same chromosome was observed in several studies. Boone and Keitt (12) used color mutants to describe three linkage groups associated with the compatibility factor. Linkage groups for pathogenicity were also described by Bagga and Boone (7). The genes in the linkage groups studied were usually not independent unless crossing-over had occurred.

The sexual stage usually provides the primary inoculum each season; thus, the possibility is great for the development of new biotypes or races through genetic recombination. In a breeding program for scab resistance, Shay and Williams (115) found

it necessary to classify isolates of *V. inaequalis* into physiological races based on their pathogenicity to different apple cultivars. To date, five races have been identified and named according to the cultivars they infect (47, 132, 137). Race 1, the common race found throughout the United States and many other countries, attacks many apple cultivars. Race 2 is pathogenic on the cultivars 'Dolgo' and 'Geneva,' (which are resistant to Race 1), and a clone of the Russian apple, *M. pumila* nr. R12740-7A. Race 3 is pathogenic on 'Geneva,' race 4 is pathogenic on certain other clones of the Russian apple (R12740-7A), and race 5 is pathogenic on progenies of *Malus micromalus* and *M. astrosanguinea* 804, which exhibit the pit type of resistance. 'Dolgo,' 'Geneva,' two different clones of R12740-7A, 'Florina,' 'Golden Delicious,' and *M. micromalus* have been selected as the differentials for the five races of *V. inaequalis* (75).

The type of host reaction is used to determine the susceptibility of a particular cultivar. Initially, host reaction was classified as either resistant or susceptible, but it became necessary in breeding programs for scab resistance to group the host responses into classes based on the foliar symptoms: 0 = no macroscopic symptoms, 1 = minute pits, with no sporulation, 2 = chlorotic or necrotic lesions, no sporulation, 3 = necrotic or chlorotic lesions with little sporulation, and 4 = extensive lesions with abundant sporulation (113). A sixth classification, "M" was later added to identify a mixture of non-sporulating and sparsely sporulating lesions (132). Symptoms classified 0, 1, 2, (and sometimes M), are considered resistant responses to *V. inaequalis*, classes 3 and 4 are considered susceptible responses. Class 1 is also termed a hypersensitive reaction (47).

Ontogenic Resistance

Wiltshire (139)was the first researcher to investigate the differences in susceptibility between young and old apple leaves and fruit. He noted that the younger leaves of susceptible apple cultivars were more susceptible than the older leaves to controlled

inoculations with *Venturia inaequalis*. Later studies reported similar differences in scab development on young and old leaves (9, 63, 68, 88, 92, 107, 120, 139).

Ontogenic resistance, inherent in all apple cultivars, is generally regarded as increased resistance to *V. inaequalis* concomitant with an increase in leaf age. Keitt and Jones (68) stated that "each leaf passes through a period of maximum susceptibility into a stage of increasing resistance." Ontogenic resistance has also been defined as a delay in the appearance of symptoms in older leaves (46) or as a lack of sporulation and secondary stroma development (48).

It was once thought that the cuticle of the leaf accounted for ontogenic resistance. As the leaf aged, the cuticle thickened and presented an impenetrable barrier to germinating *V. inaequalis* spores. However, several studies have shown that the cuticle is always penetrated, regardless of leaf age, cultivar susceptibility, or the thickness of the cuticle (9, 48, 124, 68, 92, 139). The nutritional status of the host may affect the expression of ontogenic resistance. Johnstone (63) found that a nitrogen deficiency retarded growth and maturation of apple shoots and leaves, and as a result extended the time leaves remained susceptible to infection.

Most studies have shown similar time requirements for full development of ontogenic resistance. The effect of leaf age on infection by ascospores and conidia was first investigated by Schwabe (107). The age of the leaf was recorded as days after unfurling; daily observations of leaf emergence provided a record of leaf age at the time of inoculation. The size of expanding leaves was also recorded. Leaves that were 3-5 days old at the time of inoculation with ascospores developed the greatest number of lesions. Inoculation with conidia resulted in the greatest number of lesions on leaves 1-to 3-days-old at the time of inoculation. Ascospores infected leaves up to 13-days old compared with conidia which infected leaves up to 17-days old. The severity (lesions/cm^2 leaf tissue) of scab was greatest on leaves that were not fully unfurled when inoculated with conidia and was greatest on leaves that were 2-days-old when inoculated with ascospores. Schwabe

concluded that infection was more severe on terminal shoots than on lateral shoots, when most growing shoots were weak, or when trees were in their peak flush of growth. A later study by Gessler (46) showed that the leaves reached full ontogenic resistance by 10-12 days after unfurling, which coincided with the end of the rapid growth phase.

The number of leaves infected before full ontogenic resistance is reached varies depending on the cultivar and environmental factors that affect leaf maturation (63, 88, 107, 92, 120). Moore (88) noted that leaves further than the fifth leaf from the shoot tip were resistant in MM 109 seedlings. The youngest leaves were furled and, thus, were less accessible to infection. A total of six leaves, three unfurled and three unfurling, were usually infected, but the leaves were most susceptible just after becoming unfurled. Johnstone (63) inoculated shoots that had five leaves and found that although infection occurred on all five leaves, only the three youngest leaves had extensive scab development. Differences in the number of leaves infected on three different cultivars were noted by Nusbaum and Keitt (92). The three youngest leaves on 'Yellow Transparent' shoots developed macroscopic symptoms nine days after inoculation. The two youngest leaves on 'Fameuse' shoots developed lesions at 12 days, but the lesions became bronzed and did not sporulate. No lesions developed on any leaves of 'Fameuse' that were ten days old or older. On 'Missouri Pippin,' the two youngest leaves developed lesions at 14 days, and the third and fourth leaves developed lesions at 18 days.

Szkolnik (120) reported differences in the number of infected leaves of thirty cultivars in greenhouse and field studies. In the field, only the three youngest leaves on a shoot were infected compared to eight or more infected leaves on shoots under greenhouse conditions. Orchard trees matured more quickly than greenhouse-grown trees. The leaves thickened and darkened more quickly in the field than in the greenhouse, and the greenhouse trees produced twice as many new leaves per week as did the field trees. The exposure to the environmental extremes encountered in the field most likely accounted for the rapid maturation of the orchard trees, and this may have accounted for some of the

differences in susceptibility between the greenhouse and orchard trees. However, it is possible that differences in inoculum densities in the two studies could account for the differences in susceptibility. Szkolnik noted that the severity of scab (expressed as number lesions/cm^2 leaf tissue) decreased with leaf age in the greenhouse studies and that the number of leaves infected varied with the cultivar. In all cultivars, the youngest leaf averaged 7.1 lesions/cm ^{2} leaf tissue, and the second third, fourth, and seventh leaves averaged 4.5, 2.4, 1.1, and 0.1 lesions/cm^2, respectively. Most cultivars produced sporulating lesions, but on several cultivars spore production was sparse or nil. Lesion density and the number of spores per lesion are important measures of susceptibility, particularly in regards to the buildup of secondary infections.

Several studies have investigated ontogenic resistance in scab resistant cultivars. Biehn et al. (9) studied ontogenic resistance in *Malus atrosanguinea* 804 which carries the V_m (pit-type resistance) gene. The three youngest leaves exhibited the pit-type (hypersensitive) response. There was no hypersensitive response on any leaves older than the third leaf on slow-growing shoots or older than the sixth leaf on rapidly-growing shoots. The authors hypothesized that an unknown biochemical factor associated with the rapidly expanding leaf tissue influenced resistance.

Gessler and coworkers (46, 48, 124) compared stromata development in the susceptible cultivar 'Golden Delicious' and the resistant cultivar 'Liberty.' The formation of a primary stroma was affected by leaf age but not by cultivar. Primary stromata in old 'Golden Delicious' leaves (fourth leaf or older, or 12 + days old) or young, genetically resistant leaves reached only one-third the thickness attained by stromata in young susceptible 'Golden Delicious' leaves.

Ontogenic resistance apparently occurs in all cultivars. This has led to the assumption by most researchers that ontogenic resistance is stable. Gessler (46) stated that ontogenic resistance..."is true stable resistance." However, there is some evidence that ontogenic resistance does indeed "break-down" during each growing season. A study by Keitt and

Jones (68) showed that lesions did eventually develop from latent infections on older leaves that would usually have been assessed as having ontogenic resistance. The plants were exposed to a single inoculation of *V. inaequalis* conidia and then maintained in the greenhouse; therefore, all lesions which developed resulted from the one inoculation. The youngest leaves of 'Fameuse' or 'Wealthy' developed lesions 13-16 days after inoculation. Few additional lesions developed on these leaves after twenty days. Older leaves, however, did not develop lesions until 55-57 days after inoculation. On the cultivar 'Fameuse' there was a delay of 35 days for lesion development between the fourth and fifth leaves (measured from the youngest leaf at the time of inoculation). Because lesion development was not recorded much beyond 30 days after inoculation, the breakdown of ontogenic resistance has rarely been observed in inoculation studies. Olivier and Lespinasse (94) noted that there was a change in the susceptibility of host organs under field conditions. The older leaves which had been resistant to scab infections at the beginning of the summer became susceptible again at the onset of senescence, particularly in the cultivars 'Golden Delicious' and 'Starkrimson.' Olivier and Lespinasse also noted that the fruit could be attacked at the onset of maturity. They hypothesized that a physiological change took place in the leaves and fruit which once again made them more susceptible to scab infections.

The mechanisms of ontogenic resistance are poorly understood. Gessler and Stumm (48) hypothesized that a substance inhibitory to *V. inaequalis* was responsible for the decrease in stroma size in ontogenic and genetically resistant leaves. Later research provided evidence of a proteinaceous substance which is inhibitory to fungal cell wall degrading enzymes. This proteinaceous inhibitor may be responsible for ontogenic resistance (46, 124).

If ontogenic resistance does break down with the onset of senescence, this could impact significantly on the epidemiology of apple scab. As leaves once again became susceptible to latent infections, they could also become susceptible to new infections during

late season infection periods. The combination of "early" season lesion development and "new" late-season infections would increase the likelihood of two compatible mating types being present on one leaf, and this would increase the chance of fertilization, and subsequently increase the primary inoculum available for the following primary scab season.

Overwintering of *V. inaequalis*

The saprophytic stage of *V. inaequalis* is initiated shortly after leaf-fall (68, 128, 138) when the mycelium begins to ramify tissues. The formation and early development of pseudothecia follows, and by 45 days the lumen of pseudothecia are filled with pseudoparaphyses. Development during the winter months is evident mainly by increased diameter of the pseudothecia (59). The development of asci and ascospores begins in the spring and continues until nearly all the ascospores have matured. Ascospores are the sole or major component of the primary inoculum in most apple growing regions of the world. Difficulty in controlling apple scab is proportional to the number of ascospores present during the primary season (8). Therefore, to implement successful control measures it is important to understand what factors may influence the development and maturation of the pseudothecia.

Wilson (138) was one of the first to study the influence of the environment and other factors on pseudothecial development. He identified five factors that were important: the time when infection occurred, apple cultivar, time of leaf-fall, temperature, and moisture. Several other factors have since been identified by various researchers: lesion density at leaf-fall, leaf age at time of infection, inoculum concentration, the isolate of *V. inaequalis*, and the influence of other microorganisms.

The time of year when infection occurs influences the extent a leaf surface is infected, the type of lesion that develops, and, indirectly, the age of the leaf when infected. Early-

season infections commonly result in discrete-margined lesions that usually occur on the upper surface of the leaves (138). Late-season infections commonly develop as diffuse-margined lesions that occur predominantly on the lower surface of the leaf (22, 138). Wilson observed that pseudothecia developed only at the edge of discrete-margined lesions, but were throughout diffuse-margined lesi ns. He also noted that pseudothecia did not develop further than one centimeter from the margin of a lesion. If they did, they probably developed from infections not evident at the time the leaf was collected. Wilson concluded that lesion type and density at leaf-fall influenced the pseudothecial density: diffuse-margined lesions produced more pseudothecia than discrete-margined lesions, and an increase in lesion density was associated with an increase in pseudothecial density. Cook (22) confirmed these findings. Pseudothecial densities were highest on leaves infected in Jul or Aug as long as the leaves remained on the tree until Nov. Jeger (60) stated that late infections, particularly those on the lower leaf surface, were important sources of inoculum because they yielded greater numbers of pseudothecia regardless of the leaf surface exposed during the winter.

Jeger (60) did not find any relationship between lesion density and the rate of pseudothecial development or pseudothecial density. However, he assessed only leaves with discrete-margined lesions, so this restricted sampling of infected leaves did not include pseudothecial development on leaves which had diffuse-margined lesions or lesions of different ages. The diffuse-margined lesions that develop late in the season most likely arise from multiple infections on a leaf, and this would increase the likelihood of fertilization between sexually compatible strains of the fungus (22). Gadoury and MacHardy (42) reported that low disease incidence (< 5% of the leaves infected) and severity (1-2 lesions per leaf) affected the pseudothecial density. In a low inoculum orchard, lesion density can influence the number of lesions that will become fertile. Hirst and Stedman (56) found a significant correlation between ascospore productivity and disease incidence ($r = 0.691$, $p = <.01$).

Leaf-fall. Keitt and Jones (68) were the first to report a relationship between the time of leaf-fall and the time of maturation of pseudothecia. Wilson (138) later noted that extensive ramification of the lamina did not occur until the leaves were killed. Leaves that remained on the tree throughout the winter did not produce pseudothecia. In general, the earlier the leaves were removed from the trees the earlier the ascospores matured. The relationship was not consistent however, because leaves that were removed two months earlier than others matured only four days earlier. Hirst and Stedman (56) reported that the time of ascospore maturation was delayed by late leaf abscission, but Wilson (138) noted that a delay of leaf-fall shortened the time needed for ascospore maturation. Leaves collected in Aug took 253 days to mature, while leaves collected in Dec took only 175 days to mature. The influence of leaf-fall on pseudothecial maturation and development was studied under controlled temperature and moisture by Louw (79), and in general his laboratory data supported the field data. The number of days between subsequent dates of leaf-fall were not proportional to the number of days needed to reach maturity, but pseudothecia did mature in the same sequence as the leaves on which they formed fell from the trees. Leaves collected on 21 Apr, 1 May, and 8 May produced mature ascospores 112, 102, and 95 days later, respectively. According to Cook (22), the longer a leaf had been infected when abscission occurred, the higher the pseudothecial density. The highest pseudothecial densities occurred on leaves artificially inoculated in Jul and removed in Nov. Burchill and Hutton (21) and Hirst and Stedman (55) also reported that the greatest number of ascospores was produced on leaves that had abscised in late autumn. The date of leaf-fall did not appear to influence pseudothecial density in a controlled environment study by Gadoury and MacHardy (38). Pseudothecial density was 41, 48, and 43 per cm^2 leaf tissue on leaves collected on 15 Aug, 12 Sep, and 10 Oct, respectively, indicating the time of leaf-fall was unrelated to pseudothecial density.

Cultivar. Commercial apple cultivars differ in their susceptibility to scab, and these differences may result in differences in lesion density. Cultivars may also differ in the time

their leaves fall. Wilson (138) compared the rate of pseudothecial maturation in leaves of 17 apple and crabapple cultivars that had similar amounts of infection. The pseudothecia of some cultivars, most notably 'Virginia Crabapple,' matured more quickly than those produced in leaves of the other 16 cultivars. The crabapple leaves became pliable more quickly after wetting than those of other cultivars, and this suggested to Wilson that it was possible that leaves that would wet the easiest would have the most rapid pseudothecial development because the pseudothecia could absorb and hold moisture better, particularly in dry autumns and springs. Baines (8) compared the rate of ascospore maturation in leaves from eleven cultivars picked on 2 Nov. By 2 Mar, from 1-30% of the pseudothecia in all cultivars had mature spores, and by 17 Mar mature spores were observed in 33-98% of the pseudothecia from all cultivars. However, the variation in maturation rates was as great within cultivars as it was between cultivars; therefore, the differences in maturation rate were believed to be of no practical significance.

Ascospore productivity was compared on heavily infected leaves collected from six cultivars in Oct and overwintered in the orchard (87). Ascospore productivity and rate of maturation were determined weekly by discharging ascospores into water in petri dishes and counting the number of ascospores per low-power microscopic field. Ascospore productivity differed as much as 20-fold between some of the cultivars, but the rate of ascospore maturation did not differ between any of the cultivars. Ascospore productivity ranged from 2 ('Ruby') to 23 ('Winesap') ascospores per microscope field. The peak ascospore discharge for all cultivars occurred on leaves examined on 19 Mar. The stage of tree growth at peak ascospore productivity ranged from 1.27 cm (1/2") green tip ('Winesap') to pink ('Summer Red' and 'Granny Smith').

Jeger et al. (62) reported that the 50 to 100-fold differences in ascospore yield on the ten cultivars they studied were due to differences in the rate of pseudothecial developed among the cultivars. Differences in the rate of pseudothecial development were noted primarily at the green tip stage of tree development. Little variation in pseudothecial density

or ascal density was noted among the cultivars. The researchers felt that the variation in pseudothecial and ascal densities among cultivars was not great enough to account for the differences in ascospore productivity reported by Moller. They stated that differences in maturation rates accounted for the differences in ascospore productivity. It is not clear how Jeger and his co-workers came to this conclusion given the evidence from Moller's work; all cultivars followed the same maturation pattern, with peak ascospore productivity occurring during the same week of sampling. If maturation rates had differed, then the peak ascospore productivity should have occurred at different times for each cultivar. Support for Moller's conclusion was provided by James and Sutton (59) who did not find any significant differences in the maturation rate of pseudothecia from leaves of four different cultivars sampled at two dates.

Gadoury and MacHardy (42) did not find any differences in pseudothecial density per lesion or ascal density per pseudothecium in leaves from three cultivars. The number of pseudothecia per lesion were 16, 21, and 13, and the numbers of asci per pseudothecium were 101, 103, and 111 for 'McIntosh', 'Cortland', and 'Delicious', respectively. Cultivars that differ in their susceptibility to scab may have different levels of scab incidence and severity, and an increase in incidence and severity may result in an increase in ascospore productivity. Although leaves were assessed in the Moller (87) and Jeger et al. (62) studies, actual lesion densities were not determined. Therefore, it cannot be assumed that the differences in ascospore productivity they reported among the cultivars were accurate measures of the capacity of *V. inaequalis* to produce pseudothecia on each cultivar.

Isolate and Inoculum Concentration. The inoculum concentration can also influence the overwintering stage. O'Leary and Sutton (93) reported that the number of pseudothecia was significantly correlated ($r = 0.82$, $p = <0.01$) with the inoculum concentration. The inoculum consisted of four conidial isolates (two of each mating type) that were mixed and applied at 3, 6, and 12 x 10^4 conidia/ml. As discussed earlier, the increase in the number

of pseudothecia that accompanies an increase in lesion incidence or severity is most likely due to an increase in the probability of fertilization between two compatible mating types.

The isolate of *V. inaequalis* and the cultivar which serve as the source of the inoculum can affect the pseudothecial density and ascospore productivity. Palmiter (98) demonstrated that isolates from different cultivars produced different numbers of pseudothecia in culture. Parasitic fitness may also determine which isolates will predominate in an orchard. In an orchard where one cultivar is predominant, the fungal strain most capable of attacking that cultivar will persist and multiply (22, 128, 129). This aspect of inoculum source is less important in mixed-planting orchards because the inoculum from several cultivars would always be mixed, with the result that no one strain would predominate in the orchard.

Temperature. Keitt and Jones (68) reported that temperature has a major effect on ascospore maturation. The rate of ascospore maturation increased as temperature increased from 12 to 20 C, but decreased as temperatures increased above 24 C, and no development occurred above 30 C. The optimum temperature was 20 C. In Wilson's (138) study, the optimum temperature for initiation and growth of pseudothecia was between 13-16 C. Maturation of ascospores occurred most rapidly at 18 C, and no spores were formed at 28 C. However, if the leaves were kept dry when the temperatures were above 24 C the ascospores were not killed. Other studies, supporting the findings of Wilson, have also reported differences in the optimum temperatures for the initiation and the maturation of pseudothecia, (38, 59, 61).

Temperature during the first 28 days after leaf-fall has the greatest influence on the number of pseudothecia that developed (38). There was an inverse relationship between an increase in temperature and the number of pseudothecia formed. The number of asci that reached maturation was determined by the temperatures that occurred in late winter to early spring between the time of the first appearance of ascus initials and the first appearance of delimited ascospores (43). In one study, Louw (79) indicated that the temperatures

necessary for pseudothecial initiation and maturation were very similar, which was in contrast to the findings mentioned above.

Moisture. Excessive moisture and drying appear to be the limiting factors in pseudothecial development and maturation (59). When leaves were kept dry after leaf-fall no pseudothecia developed in any of the leaves (59, 61, 79, 138). Prolonged wetting however, usually caused ascospores to develop abnormally or to abort (79, 138). Intermittent wetting appeared to provide the optimum moisture requirement for pseudothecial development and maturation (68, 138). Although dew does not provide enough moisture for ascospore discharge, it does appear to provide adequate moisture for pseudothecial development and maturation (68, 138). Prolonged dry periods in the spring can delay ascospore maturation and productivity (55, 59, 79). Combined high temperatures and excessive moisture are particularly detrimental to ascospore maturation, and in North Carolina ascospores were reported to abort when exposed to these conditions (93). In England, pseudothecia were most abundant when Nov rainfall was high and the temperatures were low (61).

Geotropic Responses. Reports from two studies provide conflicting evidence concerning the geotropic response in developing pseudothecia. Jeger (60) did not find significant evidence of geotropism. If lesions were on the lower leaf surface, pseudothecia developed on whichever surface was exposed during the winter, but if lesions were on the upper surface, more pseudothecia developed on that surface regardless of the surface exposed. In contrast, Gadoury and MacHardy (41) reported that a strong geotropic response was present. When leaves with lesions on the upper surface were overwintered, all of the pseudothecia that formed occurred on whichever surface faced upward. The leaf surface bearing the lesions had very little influence which side pseudothecia formed. Approximately 70% of the leaves developed pseudothecia oriented toward whichever surface faced upward. Biostiolate pseudothecia and pseudothecia on both surfaces developed if the leaves were overturned during the development period. It appears that

negative geotropism is a mechanism that ensures that ascospores will be released into the air regardless of the surface exposed during the winter. Leaves collected by Jeger were severely infected and came from a high inoculum orchard. This would increase the likelihood of inconspicuous late-season lesions developing, making accurate assessment of infection difficult, and this may explain the discrepancy with the study by Gadoury and MacHardy (41).

Influence of Phylloplane Microorganisms. Several studies have examined the influence of phylloplane microorganisms on the development of *V. inaequalis*. Cook (22) reported that a suspension of microflora obtained by dipping overwintering leaves in water suppressed pseudothecial development on leaf discs (but not on whole leaves). High numbers of *Fusarium sp.* and *Cladosporium* spores were recorded, particularly on leaves treated with 5% urea. *Athelia bombacina* and *Chaetomium globosum* reduced the number of pseudothecia reaching maturation by 100% and 40-90%, respectively (51). Pseudothecia did not mature past the stage where the lumen was filled with pseudoparaphyses when treated with a suspension of *A. bombacina* (140). There have been no studies to determine if saprophytes exhibiting "biocontrol" of apple scab are more abundant on leaves from certain apple cultivars.

Ascospore Productivity

The production of ascospores by *Venturia inaequalis* has been monitored in apple scab management programs for decades (24, 68). The abundance or scarcity of the primary inoculum (ascospores) has a direct effect on the number of scab infections developing in an orchard during a single growing season (21). Measurements of ascospore release during the growing season provide information on the initiation, duration, and termination of the primary scab season while allowing relative comparisons of productivity between years and between orchards or cultivars (49, 54, 83, 87, 108)

The terms 'relative ascospore dose' and 'ascospore productivity' were introduced by Hirst in 1952 (53). Relative ascospore dose, expressed as the number of spores per volume orchard air (m^3), is the total hourly estimates of ascospores trapped for the year, and represents the total ascospores to which trees in the orchard were exposed. Ascospore productivity, also referred to as ascospore potential, is the total number ascospores produced per cm^2 leaf tissue, and was determined by Hirst by sequentially sampling leaf tissue at weekly intervals until the supply of ascospores was exhausted. Both terms are relative in that the traps were designed to compare yields rather than the total number of ascospores released (55).

Estimates of ascospore dose have been based on estimates of pseudothecial density (pseudothecia per unit leaf area) or on the number of ascospores trapped per (m^3) of orchard air. Curtis (24) calculated a potential of 64,000 ascospores produced from a single leaf containing 200 pseudothecia, assuming 40 asci per pseudothecium and eight ascospores per ascus. The calculated potential ascospore dose was much higher in poorly maintained orchards where pseudothecial density was as great as 1,300 pseudothecia per sq inch leaf area. Curtis also determined ascospore dose by placing an adhesive-coated slide over each of five leaves exposed to rain. The slides were replaced daily until all ascospores were exhausted. Total ascospore productivity for each leaf was 3,739; 3,082; 4,905; 432; and 6,272, respectively.

Keitt and Jones (68) were the first to collect ascospores from the orchard air using a suction spore trap that sampled air at one cu ft (0.0283 m^3) per minute. The greatest airborne density of ascospores they recorded was 289/cu ft air (.0283 m^3) for a five hour period. The development of the volumetric spore trap by Hirst (53) allowed more efficient sampling. Using the Hirst spore trap at 0.5m above ground level, Miller and Waggoner (85) trapped 21,600 ascospores/m^3 air during a single rainy period. Hirst and Stedman (54) sampled orchards of 'Bramley's Seedling' with traps placed 2 m above ground level. The highest ascospore dose for a single orchard was 38,500 Ascospores/m^3 air, where the

greatest density of airborne ascospores collected in one hour was 4,000/m^3. Yearly comparisons of ascospore dose showed variation as great as a 54-fold difference for the same orchard. Differences between orchards were as great as 30-fold in a single year.

Ascospore productivity has been estimated from spores discharged from moistened leaves and expressed as ascospores/cm^2 leaf tissue. Hirst and Stedman (55), using quadruple ascospore liberation tunnels, determined productivity from leaves of 'Bramley's Seedling' that were subjected to successive weekly wettings until the supply of Ascospores was exhausted. Results from six orchards varied form 6 to 2,493 ascospores/cm^2 leaf tissue in 1956. Schwabe and Matthee (108) reported productivities of 2,000, 13,000, 750, and 2,500/cm^2 leaf for 1966, 1968, 1969, and 1970, respectively, indicating that ascospore productivity may vary considerably from year-to-year in a single orchard. Using a spore tower, Gilpatrick and Szkolnik (49) found an 11-fold difference in ascospore productivity between orchards in two areas of New York state.

The length of time ascospores are released during a single wetting period and the total duration of pseudothecial productivity (primary scab season) have also been investigated In one field study (55) 90% of the ascospores discharged from a single wetting were trapped between 1.5 to 5 hr after the rain began, depending on the temperature (43F-60F). In a laboratory study, peak ascospore release occurred approximately two hours after the start of a wetting period, with 75% of the ascospores released within 3-6 hours during prolonged wetting periods (49). In another field study (128), the primary scab season lasted for approximately one month: the first mature spores were released on 4 May and pseudothecia were empty on 6 Jun. In several studies, the duration of the primary scab season was 5-9, 12, 8-12, 10, 16, and 12 weeks (16, 49, 54, 68, 108, 119). In all of these studies, Ascospores were first trapped near bud break, and the peak ascospore dose occurred between early bloom and petal fall. The end of the primary season for scab management purposes is more difficult to determine than the beginning of the primary season because different methods (e.g. squash mounts, spore trapping) of assessing

ascospore maturation often give different results. Although a small percentage (5-7%) of the season's mature ascospores may be found in pseudothecia well into late Jun or early Jul, it does not appear that the spores would have any significant impact on the development of primary scab. Environmental conditions are often unfavorable, and the ascospores usually are not discharged out of the leaf litter (85). Gilpatrick and Szkolnik (49) estimated the end of the primary season at six weeks after petal fall, and Hirst and Stedman (54) stated the end of the primary scab season occurred with the thirtieth day of measurable rainfall from bud break.

Moller (87) compared ascospore productivity on six cultivars in a mixed planting that had been heavily infected with scab the previous growing season. Differences in productivity among the cultivars was as great as 20-fold. The phenological stage of tree growth during which peak productivity occurred varied depending upon the cultivar. Although leaves collected were similarly heavily infected, it is not known how much the ascospore productivity/lesion differed among the cultivars because actual lesion density was not determined. Therefore, differences in lesion densities could have accounted for the differences in ascospore productivity. Regardless, the results suggest that cultivar should be considered when implementing apple scab management programs.

Several studies reported that extended dry periods allowed large numbers of mature spores to accumulate, accounting for the high numbers of ascospores trapped during wet periods immediately following long dry periods. (17, 49, 108). Extended dry periods may also cause an increase in the length of the primary scab season, presumably by slowing the rate of ascospore maturation. Schwabe and Matthee (108) found that the ascospore supply was exhausted in twelve weeks provided the leaves were wetted at weekly intervals, but longer intervals between wettings extended the primary scab season. They also found a relationship between lesion age and ascospore productivity. Lesions that developed late in the growing season produced three times as many ascospores as did lesions formed early in the season.

Ascospore dose is determined by many factors including cultural and control practices, apple cultivar, severity and timing of foliar infections the previous year, environmental factors during the autumn and spring, and the amount of infected leaves remaining in the leaf litter in the spring (49, 54). The term potential ascospore dose (PAD) was introduced by MacHardy and Jeger (83). Although PAD did not include all of the above mentioned factors, PAD did account for most of the factors influencing productivity up to the beginning of the primary scab season. PAD is the estimated total seasonal ascospore production per m^2 orchard floor, and is the product of lesion density at leaf fall, pseudothecial density, ascal density, ascospores per ascus, and leaf litter density at bud break. An investigation by Gadoury and MacHardy (42) led to the development of a working model to forecast PAD in commercial orchards. The workers based their calculations on studies involving the cultivars 'McIntosh,' 'Cortland,' and 'Red Delicious.' Statistical differences were not found between any of the cultivars for several components of the model, so "standards" were calculated and inserted into the PAD formula for those components: pseudothecial density was 21.6 pseudothecia per lesion, ascal density was 122 asci per pseudothecium, and leaf area per terminal (used in calculating lesion density) was 430 cm^2. Thus, the only measurements needed to determine PAD were the total number of lesions for 600 shoots at leaf-fall and the leaf litter density at bud-break. The model has proven useful in forecasting ascospores dose at the beginning of the growing season and in comparing the effectiveness of control strategies. However, the model does have shortfalls. Lesion density is based on discrete lesions and does not account for pseudothecial densities in diffuse margined lesions. Also, the ascal density, pseudothecial density, and leaf area per shoot is not known for apple cultivars other that 'McIntosh,' 'Cortland,' and 'Delicious,' and these components could vary considerably from the "standards" used in the model.

The Conidia

Spilocea pomi Fries, the conidial stage of *Venturia inaequalis* produces the secondary inoculum which is responsible for the buildup of apple scab in the orchard in the late spring, summer, and fall. The conidia are formed initially on lesions that develop as a result of infections initiated by ascospores (primary inoculum) in the early spring. Foliar lesions become visible macroscopically when the conidia rupture the cuticle. The conidia are disseminated primarily by splashing rain, but may be carried short (10 m) distances during rains accompanied by strong winds (36, 68, 129). Strong winds alone are inefficient at dislodging conidia from their conidiophores A few workers however, have trapped conidia from the orchard air on dry days (54, 118, 129). The conidia were most likely dislodged by leaves whipping against each other or by drops of dew falling from leaves onto lesions on leaves below.

The rate at which conidia germinate and the proportion of germinated conidia that penetrate the leaf cuticle are determined largely by temperature and the duration of wetness. Germination occurs only in the presence of free water from 1 C to 32 C, with an optimum of 20 C (50, 64, 68, 78, 117, 122). Temperatures greater than 32 C inhibit conidial germination. Louw (77) and Keitt (64) reported an optimum temperature of 19 C for infection and incubation. The incubation period lasted 17-19 days at 8 C and nine days at 19 C. No further development of the fungus occurred at temperatures above 25 C in either study. Sporulation on lesions occurred from 4 to 28 C, with an optimum of 6 to 20 C (117). Maximum sporulation occurred at a relative humidity of 90%, and was reduced by 50% at a relative humidity of 100% or 80%. The greatest rate of lesion expansion occurred at 19 C, with little lesion expansion at 0 C or at greater than 26 C.

Lesion expansion and conidium production on a lesions ceases in approximately 30 to 36 days after inoculation. Nusbaum and Keitt (92) reported that center of the lesions became necrotic around 19 days after inoculation. The necrotic area continued to expand

until conidium production ceased 30 to 36 days after the lesion had appeared. Johnstone (63) investigated the relationship between lesion age and the viability of conidia on the lesion. 'Morgan Sweet' leaves with secondary lesions initiated during a single infection period were sampled for conidium viability during Sep and Oct. Viability decreased from 94% on 19 Sep to 65% on 10 Oct and 53% on 25 Oct. Johnstone hypothesized that the decrease in viability was caused by an increase in the proportion of old, desiccated spores and an increase in surface contaminants, particularly yeasts. A study by Keitt and Jones (68) however, provided evidence that conidia could survive through the fall and winter if the leaves were kept dry. Heavily scabbed leaves were protected from wetting in a standard instrument shelter. Germination of conidia was 99, 10, 50, 70, 50, 70, 80, and 80% on 2 Jul, 5 Aug, 8 Sep, 6 Oct, 5 Nov, 3 Dec, 7 Jan, 21 Feb, and 1 Apr, respectively. No viable conidia were found on lesions on leaves that had overwintered in the orchard under natural conditions

Few studies have investigated conidial productivity in a lesion (relative number conidia/cm^2 lesion area). In one study 300,000, 155,000, 82,000, 53,000, and 50,000 conidia/cm^2 lesion area were collected from lesions on 'Rome Beauty,' 'Delicious,' 'Golden Delicious,' 'Stayman,' and 'Cortland' leaves, respectively, on 31 May, but the variability in conidial production among lesions on the same cultivar was so great that differences in conidial productivity among the five cultivars were not significant (52). Variations in the age of the lesions may have accounted for the differences in conidial production from lesions within a single cultivar. Szkolnik (120) conducted a field and greenhouse study on conidial productivity in 30 apple cultivars. Infected leaves were grouped by leaf age and spores were washed from the lesions and counted to determine the number of conidia/lesion. The number of leaves infected varied with the cultivar, but the four youngest leaves were infected in all the cultivars; therefore, the conidium productivity was determined from the four youngest leaves for each cultivar. Conidium productivity in the greenhouse ranged from 185,000 conidia/lesion on 'Spigold' to 20 conidia/lesion on

'Priscilla'. In the field study, spore numbers were higher on 'Spigold' (514,000 conidia/lesion), and no conidia were found on 'Priscilla.'. Lesions sampled in the greenhouse study were 2 to 3 weeks old , but lesion age was not known for the field study. Both studies used destructive sampling methods. The use of a non-destructive sampling method would have allowed the relative conidium production to be determined for a single lesion for the entire growing season. This would also provide information on lesion longevity (period of viable conidia production). Information on relative conidial productivity and lesion longevity could prove useful in determining relative susceptibility to scab among apple cultivars.

Incubation Period

The term 'latent period' refers to the period between infection and the first appearance of macroscopic symptoms. The length of the latent period is influenced by leaf age, temperature, and relative humidity. Ontogenic resistance is primarily responsible for the lengthening of the latent period on progressively older leaves.

In an early laboratory study by Wallace (128), lesions first appeared on seedlings between 8 to 15 days after inoculation with ascospores. It was not until 1926 however, that the relationship between temperature and the length of the latent period was established (64, 68). Keitt (64) reported that the incubation period for seedlings inoculated with ascospores ranged from 17 days at 8 C to 8 to 12 days at 20-25 C. At 26 C, either sparse symptoms or no symptoms developed after 13 days. Keitt concluded that the effect of temperature during incubation closely paralleled the effect of temperature during infection. The effect of intermittent high temperatures on the length of the latent period was also investigated by Keitt. He found that the fungus was not killed, but symptom development was delayed markedly after four 8 hr exposures at 31C. A 24 hr exposure at 31C did not stop eventual development of symptoms, but an exposure of 48 hr did halt the development of

symptoms. The relationship between temperature and latent period was confirmed by Louw (77). 'White Winter Pearmain' trees inoculated with conidia and incubated at 8, 12, 16, and 19C, developed lesions 18, 15, 11, and 10 days after inoculation, respectively. Lesions did not develop on trees incubated at 25C.

An orchard study by Mills (86) confirmed the relationship between temperature and latent period under orchard conditions. Mills recorded temperatures during the first major infection period for each of 27 years and found a strong correlation (corr. coef. -0.765) between the mean temperature during the latent period and the length of the latent period. The latent period ranged from 17 days at a mean temperature of 9.2C to 8 days at 18.6C. The mean temperature during the five days immediately following the infection period was also strongly correlated (corr. coef. -0.659) with the latent period which ranged from 19 days at 2.2C to 7 days at 23C. The results reported by Tomerlin and Jones (121) generally agreed with those of Mills', although they did not find a relationship between latent period and the mean temperature during the five days immediately following an infection period. There were no differences in the time required for lesions to develop on seedlings incubated for five days at 20C followed by five days at 10C and those incubated at 10C for five days followed by 20C for five days. Tomerlin and Jones also reported that exposure to low relative humidity (60-70%) for longer than six days delayed lesion development. Macroscopic examination of the leaves revealed that the subcuticular mycelium was normal in appearance yet conidia failed to develop, indicating that low relative humidity affected sporulation. The results from inoculations on 'McIntosh,' 'Golden Delicious,' and 'Jonathan' indicated that latent periods were similar among the cultivars. All three cultivars are fairly susceptible to scab, so it remains to be determined if the latent period differs for cultivars that vary in their relative resistance to apple scab.

CHAPTER II

OVERWINTERING STUDY

Introduction

Venturia inaequalis overwinters as pseudothecia in fallen infected apple leaves. The development of asci and ascospores within the pseudothecium begins near bud-break and continues until all ascospores have matured. Ascospores constitute most or all of the primary inoculum in apple growing regions of the world. The difficulty in controlling apple scab in these regions has historically been proportional to the number of ascospores present during the primary scab season. The implementation of a successful apple scab management program is dependent upon a thorough understanding of those factors which may influence the development and maturation of pseudothecia and the production of ascospores.

Few studies have compared the components of innate partial resistance that occur during the overwintering phase of *V. inaequalis*: pseudothecial density, ascal density, and ascospore productivity. Wilson (138) identified cultivar as a factor that influenced pseudothecial development. He noted that pseudothecia on 'Virginia Crabapple' leaves matured more quickly than they did on leaves of 16 other apple cultivars. Baines (8) reported variation in rate of ascospore maturation among 11 cultivars, but the variation was as great within a cultivar as the variation between cultivars. Moller (87) reported that ascospore maturation rate did not differ among six apple cultivars, but ascospore productivity differed by 20-fold. Jeger et al.(62) reported 50 to 100-fold differences in ascospore productivity among ten cultivars, but there was little variation in pseudothecial density and ascal density, so they concluded that differences in maturation rate accounted

for the differences in productivity. These studies provide conflicting data on the influence that cultivar has on ascospore productivity and the rate of ascospore maturation.

The objectives of the overwintering study were to compare nine apple cultivars for; (i) pseudothecial density, ascal density, and ascospore productivity/cm^2 leaf, and (ii) the rate of ascospore maturation of *V. inaequalis* .

Materials and Methods

Fall Leaf Collection

One hundred heavily scabbed leaves were collected from 'Delicious' (RD), McIntosh' (MC), 'Stayman' (ST), 'Mutsu' (MU), 'Golden Delicious'(GD), 'Spartan' (SP), and 'Rome' (RO), respectively in late Oct, 1989 and 1990 (excluding 'Stayman'). Leaves from 'Paula Red' (PR) and 'Ida Red' (IR) were not collected in either year nor from 'Stayman ' in 1990, because too few lesions developed during the growing season. The leaves selected for collection had a mix of older definite-margined and younger diffuse-margined lesions on the adaxial surface. After the petioles were removed, the leaves were placed in fiberglass mesh (screen) packets and overwintered with the adaxial surface exposed under natural conditions at the Mast Road orchard in Durham, NH. A sheet of 6.35 mm (1/4") hardware cloth was placed over the packets to prevent them from being disturbed by strong winds. The leaves were retrieved from the orchard on 13 Apr in 1990 and 2 Apr in 1991. Each leaf was examined with a dissecting microscope (25x) with transmitted light, and one 2.7 cm^2 disk was cut from the area of greatest ascocarp density. The procedure was repeated for a total of 30 leaves for each cultivar. Ten leaf discs were sandwiched between 30.5 cm^2 pieces of wire mesh screen and 6.35 mm hardware cloth for a total of three 'packets' for each cultivar. The packets were placed in the orchard under a wooden 'lean-to' to protect them from becoming wet by rainfall.

Ascospore Maturation Rate and Productivity

Sampling to determine relative ascospore productivity and ascospore maturation rate was initiated on 17 May 1990 and 2 May 1991. The packets were removed from the orchard, brought into the lab, and soaked in water for 20 min at room temperature (~ 22C).

The packets (and discs) were blotted and placed on volumetric "funnel-traps" with the adaxial disk surface down.

In 1990, each "funnel-trap" was placed between two 15 watt incandescent light bulbs which supplied sufficient light to stimulate ascospore release from the wetted leaf discs. The far-red light energy recorded with a radiometer at the surface of the leaf discs was 0.65 x 10^{-3} W/cm^2/nm (between 600 and 875 nm). Light within these wavelengths is reported to stimulate ascospore release (17). In 1991 the "funnel-traps" (see Appendix Fig. 1) were illuminated by GE wide spectrum lights (F20T12PL\AQ). Air drawn through the orifice at the base of the funnels was sampled at 8.17 (± .15) L/min . The leaf discs were misted with water every 10 min during the 30 min sampling interval. Ascospores drawn through the orifice were deposited on a microscope slide placed directly beneath the orifice (see Appendix Fig. 2). Each slide was removed from the traps and prepared as follows: a few drops of gelvatol (35 g gelvatol grade 40-20 + 50 ml glycerol dissolved in 100 ml distilled water) were placed on a 20 x 40 mm cover slip which was then inverted and carefully placed on the spore deposit area of the slide, taking care not to disturb the deposited ascospores. The slide was left undisturbed for 24 hr to allow the gelvatol to set.

The slides were examined microscopically (20x) and the relative number of ascospores trapped on each slide was determined by counting the spores in a total of five transects consisting of 20 fields each. The area examined on each slide was 90 mm^2. Several slides with spore deposits too dense to count accurately at 20x were examined at 40x. The number of fields examined was doubled at 40x, but the total area examined on the slide remained at 90 mm^2. Relative ascospore productivity (the total number of ascospores counted) was calculated for each cultivar and compared by analysis of variance to determine the effect of cultivar on ascospore productivity. Ascospore maturation rate was determined by plotting the cumulative % total ascospores collected on each sampling date against the cumulative degree-days (base = 0 C). Degree-days were cumulated beginning at the silver

tip growth stage of the apple fruit buds. Ascospore maturation rates for all cultivars were compared with regression analysis of probit transformed data.

Pseudothecial Density

To determine pseudothecial density (pseudothecia/cm^2 leaf tissue), each leaf disc was examined with a dissecting microscope (25x) and transmitted light. The number of fruiting structures was counted and totaled for the ten discs in each packet. Two fruiting structures were removed from each leaf disc, squashed, and examined microscopically to determine the proportion that were pseudothecia of *V. inaequalis*. The effect of cultivar on pseudothecial density was determined by comparing the pseudothecial densities for all cultivars with one-way analysis of variance.

Ascospore Maturation Rate and Ascal Density (squash mounts)

Leaves were collected from 'Delicious,' 'McIntosh,' 'Stayman,' 'Mutsu,' 'Golden Delicious,' 'Spartan', and 'Rome' in Oct 1989 and overwintered as described previously. Leaf portions were taken from the overwintered leaves on 14 April, 1,16, and 29 May, and 12 June and placed in FAA (formaldehyde/acetic acid/alcohol) to preserve pseudothecia at their stage of development at the time of sampling. Squash mounts were prepared with pseudothecia removed from 10 leaf samples from each cultivar as described by Gadoury and MacHardy (39), except that one pseudothecium rather than two was removed from each of the 10 leaf samples. Pseudothecia from ten 'McIntosh' leaves collected from the orchard floor were examined on 13, 23, and 28 Apr, on 1, 6, 8, 14, 18, 22, and 29 May, and 5, 11, 19, and 27 Jun in 1990. In 1991, 'McIntosh' leaves were sampled on 9, 16, 23, and 30 Apr, on 2, 7, 14, 21, and 28 May, and on 4 and 11 Jun. Ascospore development was determined by examining each crushed pseudothecium, recording the number of asci in each of the four maturation classes, and adjusting that number with the proper correction factor as described by Gadoury and MacHardy (39). The probits of cumulative percent mature asci (asci with matured ascospores + empty asci) were regressed against cumulative degree days (base = 0 C) to determine the rate of ascospore maturation

(33). The slopes were compared by regression analysis to determine the effect of cultivar on the rate at which ascospores matured. The mean maximum ascal densities (asci/pseudothecium) of all cultivars were compared by one way analysis of variance to determine the effect of cultivar on ascal density.

Results

Ascospore Maturation Rate

Cultivar had no significant effect (P = .05) on the rate of ascospore maturation in either 1990 or 1991. In Figs. 1a and 1b, the percent total season's ascospores trapped is regressed against cumulative degree-days . The cumulative degree-days required to reach 99% ascospore maturation was 824 in 1990 and 1125 in 1991. The probit of ascospore maturity for the pooled data was regressed against cumulative degree-days (Fig. 2). The slopes of the regression lines for the two years differed significantly (P = 0.05), indicating that the rate of ascospore maturation in 1990 was greater than in 1991. The probit of ascospore maturity for each cultivar was regressed against cumulative degree-days. The slopes of the regression lines did not differ significantly between the pooled data and that of any cultivar, indicating that the rate of ascospore maturation did not differ among the cultivars in either 1990 or 1991.

Relative Ascospore Productivity

Relative ascospore productivity differed significantly among some of the cultivars (Figs. 3 & 4). In 1990, the relative ascospore productivity ranged from 579 ascospores/cm^2 leaf tissue in 'Rome' to 180 ascospores/cm^2 leaf tissue in 'Stayman' (Fig. 3a). The relative ascospore productivity was significantly greater (P = .05) in 'Rome' than in 'Delicious,' 'Mutsu,' or 'Stayman'. The relative ascospore productivity was 23, 30, 44, 50, 65, and 69% less in 'McIntosh,' 'Spartan,' 'Golden Delicious,' 'Delicious,' 'Mutsu,' and 'Stayman,' relative to 'Rome,' respectively. In 1991, the relative ascospore productivity ranged from 425 ascospores/cm^2 leaf tissue in 'Rome' to 27 ascospores/cm^2 leaf tissue in 'Spartan,' and was significantly greater (P = .05) in 'Rome' compared to 'Delicious' and 'Spartan,' (Fig. 4a). The great variability of relative ascospore productivity within 'Golden Delicious' accounted for the lack of statistical significance when the relative ascospore productivity in 'Golden Delicious' was compared to the relative ascospore

productivity computed for the other cultivars (Appendix Fig. 3). The relative ascospore productivity was 12, 60, 79, 88, and 94% less in 'Golden Delicious,' 'Mutsu,' 'McIntosh,' 'Delicious,' and 'Spartan,' relative to 'Rome,' respectively. The number of *Leptosphaeria sp.* trapped from some of the leaf disc sets was very high, and this may have caused an error in counting the ascospores of *V. inaequalis*, particularly with the 'Spartan' samples (Fig. 5).

Pseudothecial Density

There was a significant cultivar effect on pseudothecial density in 1990. The mean pseudothecial density ranged from 27 pseudothecia/cm^2 leaf tissue on 'Rome' to 7 pseudothecia/cm^2 leaf tissue on 'Mutsu' (Fig. 3b). The mean pseudothecial density on 'Rome' was significantly greater than on 'Stayman' or 'Mutsu.' The pseudothecial density was 20%, 29%, 32%, 40%, 56%, and 73% less on 'Spartan,' 'McIntosh,' 'Golden Delicious,' 'Delicious,' 'Stayman,' and 'Mutsu,' relative to 'Rome,' respectively. The pseudothecial density varied greatly within the leaf samples of each cultivar in 1991, particularly on 'Golden Delicious' and 'Rome.' As a result, pseudothecial density did not differ significantly among the cultivars (Fig. 4b).

Approximately 50% of the fruiting bodies examined on the 'Mutsu' leaf discs were *Leptosphaeria sp.* in 1990. In 1991, 85% of the fruiting bodies on the 'Spartan' leaf discs and 62% of those on the 'Delicious' leaf discs were fungi other than *V. inaequalis*, with the majority being *Leptosphaeria sp.*

Squash Mounts

A regression line derived from the pooled data ($r^2 = .895$) was fitted to the percentage data to represent the pseudothecial maturation pattern for all seven cultivars (Fig. 6). There were no significant differences in the rate of pseudothecial maturation among the cultivars in 1990 when probit transformed data were compared by linear regression.

Cultivar did have a significant effect on ascal density (Fig. 7). Mean ascal density ranged from a high of 84 on 'Spartan' to a low of 41 on 'Stayman,' representing a 51%

decrease in ascal density. When all cultivars were compared, the mean ascal density was significantly greater on 'Spartan' than on 'Delicious' or 'Stayman.' The phenological growth stages for each cultivar when the maximum ascal density occurred were 1.27 cm (1/2") green-tip to tight-cluster ('Spartan'), 0.635 cm (1/4") to 1.27 cm green-tip ('Rome'), petal-fall to fruit-set ('McIntosh'), tight cluster ('Mutsu'), 1.27 cm green-tip to tight cluster ('Golden Delicious'), and tight cluster to pink ('Delicious' and 'Stayman').

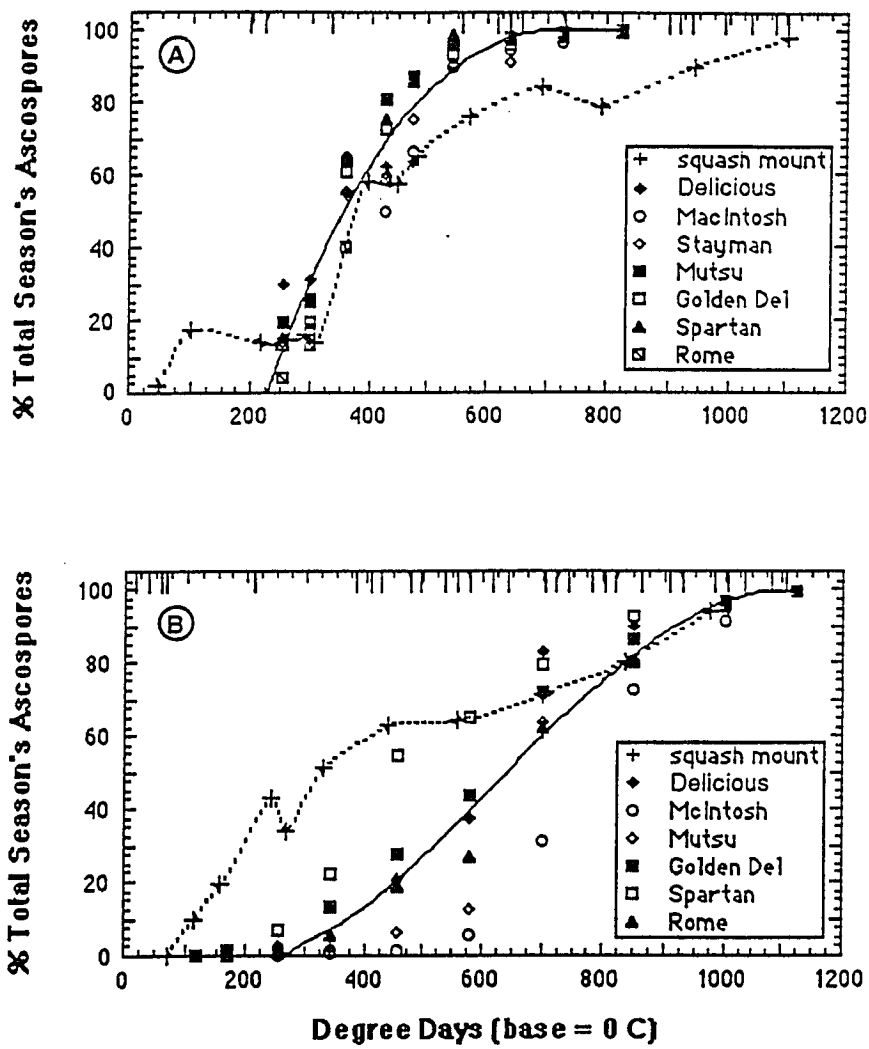


Fig. 1. Ascospore maturation determined from pseudothecial squash mounts (dotted line) and collections from spore traps (solid line) in 1990 (A) and 1991 (B). Degree days were cumulated from the silver-tip stage of tree growth. The regression line for the pooled spore trapping data is shown in each figure. Hatch marks at the top of each figure represent days with temperatures above 25 C for two or more hours.

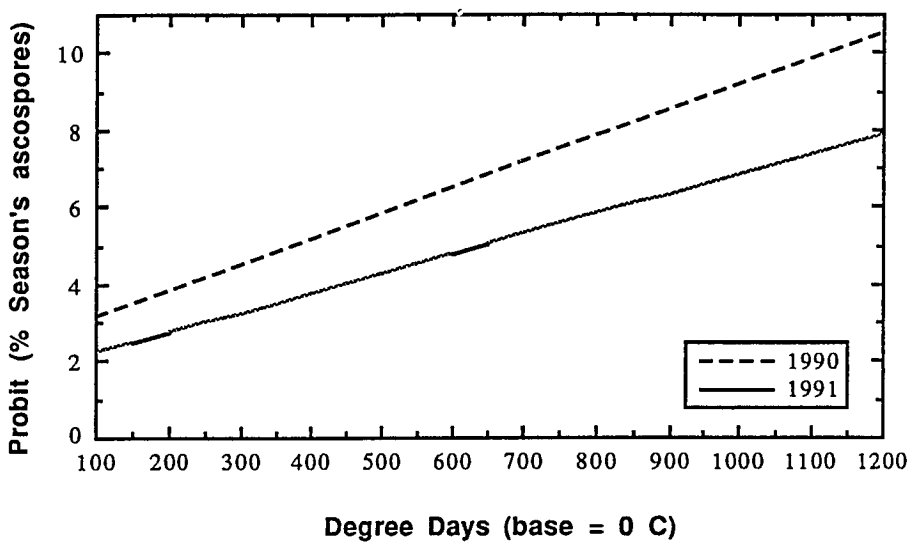


Fig. 2. Probit of the proportion of matured ascospores of *Venturia inaequalis* based on collections made from spore traps in 1990 and 1991. The regression line fitted to the pooled data for each year is shown in the graph. Slopes differed significantly (P = 0.05).

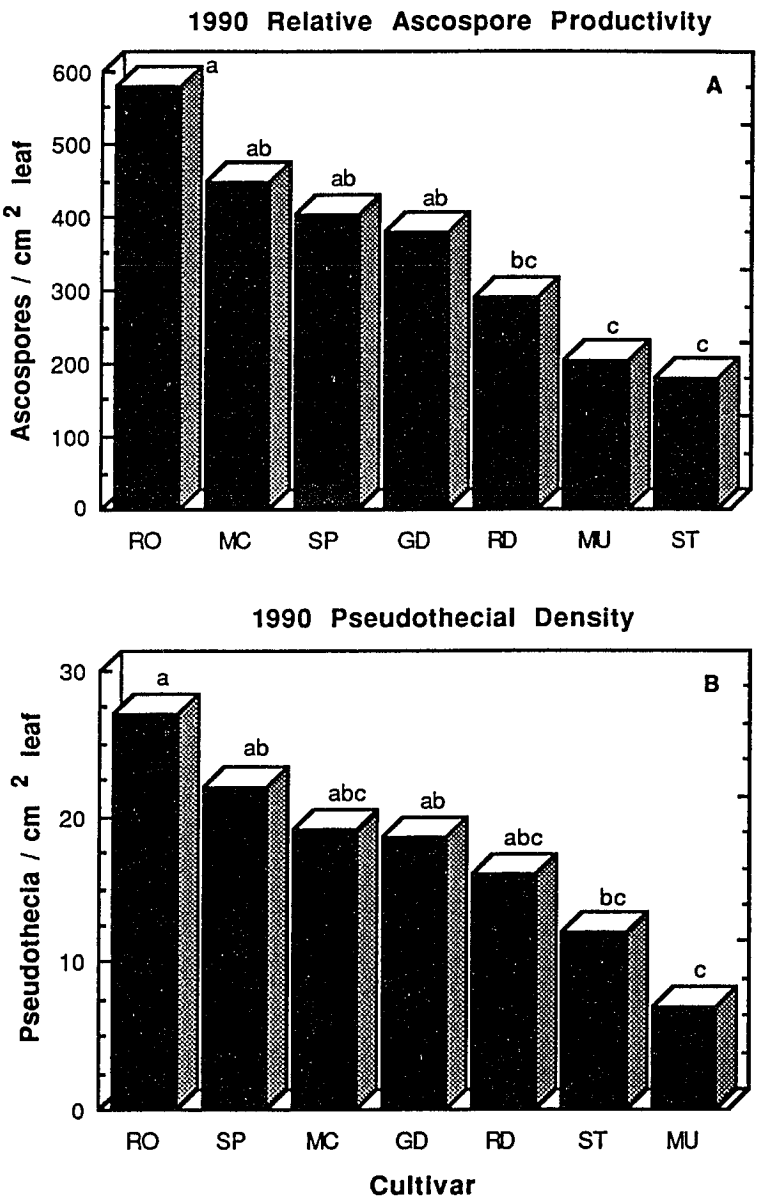


Fig. 3. Ascospore productivity (A) and pseudothecial density (B) on leaf discs infected with *Venturia inaequalis* in 1990. Means labeled by different letters differ significantly (P = 0.05). RO = 'Rome,' MC = 'McIntosh,' SP = 'Spartan,' GD = 'Golden Delicious,' RD = 'Delicious,' MU = 'Mutsu,' ST = 'Stayman.'

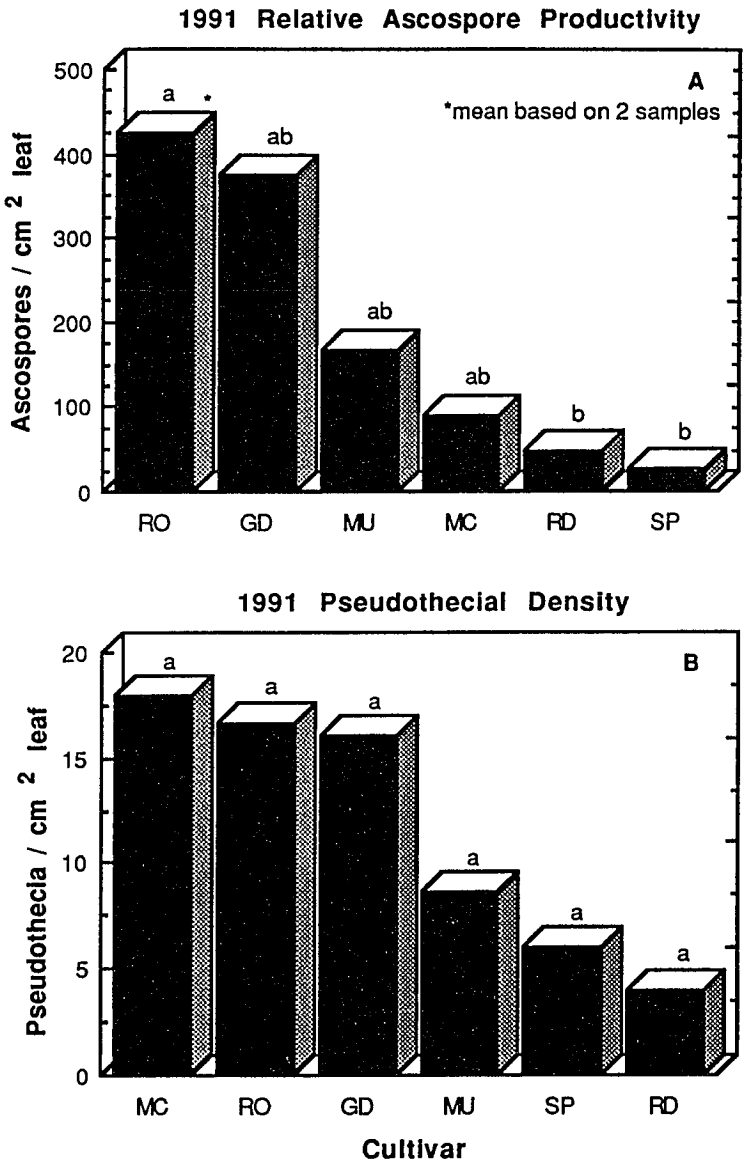


Fig. 4. Ascospore productivity (A) and pseudothecial density (B) on leaf discs infected with *Venturia inaequalis* in 1991. Means labeled by different letters differ significantly (P = 0.05). RO = 'Rome,' GD = 'Golden Delicious,' MU = 'Mutsu,' MC = 'McIntosh,' RD = 'Delicious,' SP = 'Spartan.'

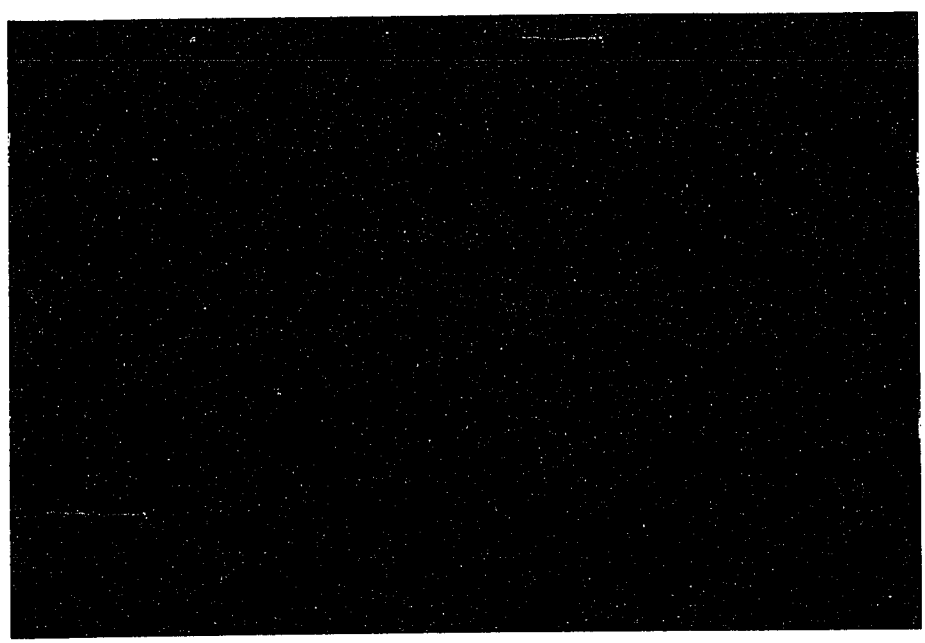


Fig. 5. Ascospores of *Leptosphaeria sp.* (200x). The spores occurred in large numbers on the slides from the "funnel-traps."

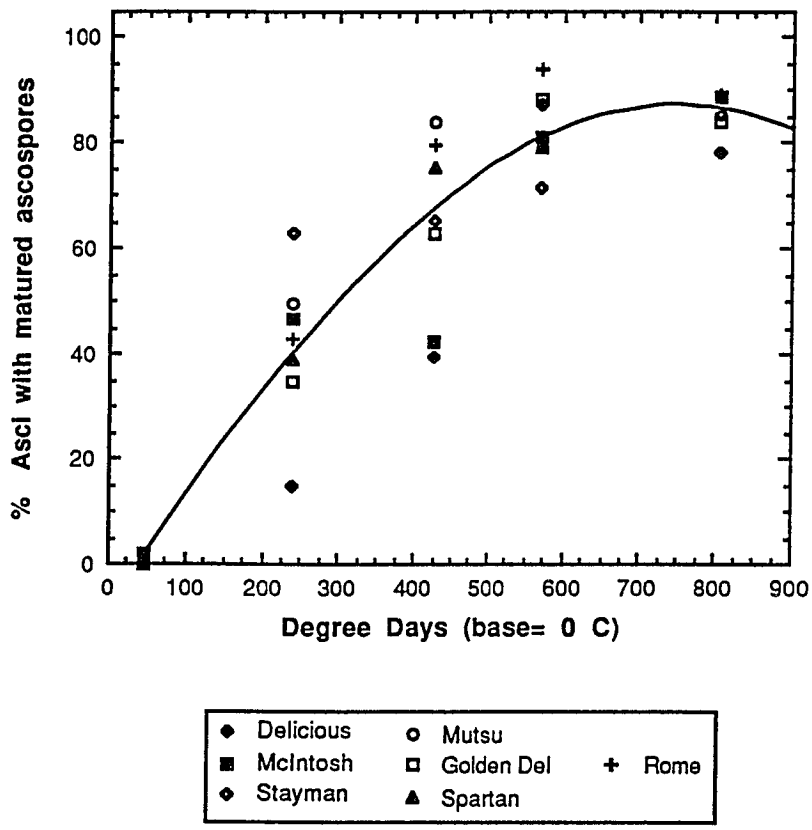


Fig. 6. Ascospore maturation in 1990 determined by microscopic examination of crushed *Venturia inaequalis* pseudothecia. The cumulative percent asci with matured ascospores plotted against degree-days accumulated from the silver-tip stage of tree growth. A single regression curve is fitted to the data for all cultivars.

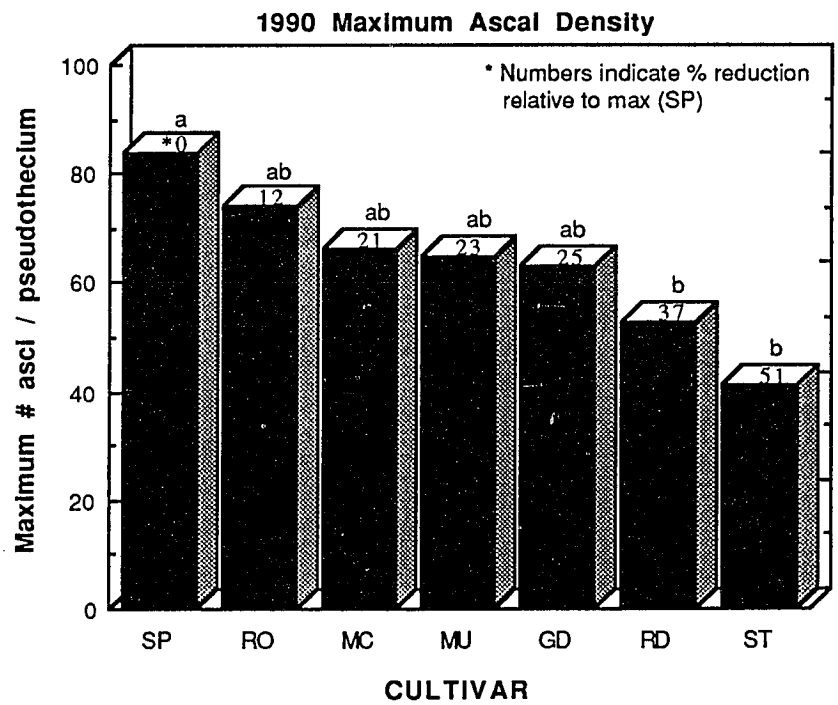


Fig. 7. Maximum ascal density in 1990 determined by microscopic examination of crushed *Venturia inaequalis* pseudothecia. Means labeled by different letters differ significantly (P = 0.05). SP = 'Spartan,' RO = 'Rome,' MC = 'McIntosh,' MU = 'Mutsu,' GD = 'Golden Delicious,' RD = 'Delicious,' ST = 'Stayman.'

Discussion

Ascospore Maturation Rate

The leaves from all cultivars were exposed to identical environmental conditions during this study. If cultivar did have an effect on the rate of ascospore maturation, that effect would have been evident as differences in the slopes of the probit regression lines (for probit transformed data) among the cultivars. There were no significant differences in the rate of ascospore maturation in either 1990 or 1991. The results agree with those reported by Moller (87), James and Sutton (59), and Baines (8) but not with those reported by Wilson (138) and Jeger et al. (62). Statistical data were not reported in the Wilson study, therefore it is not known if the figures reported for the different cultivars were different statistically. The maturation rate reported by Jeger et al. was determined by observations of pseudothecial squash mounts. When ascospore maturation is based on an assessment of squashed pseudothecia, asci that are physiologically matured are more accurately assessed when the focus is on discharged asci. This is because some asci that appear morphologically mature may not discharge ascospores upon wetting, i.e. not physiologically matured (44). However, few discharged asci were noted by Jeger et al., so it is possible that their sampling may have ended before there was ample opportunity to observe physiologically matured asci, and, as a result, their determination of ascospore maturation rate would have been inaccurate.

Ascospore maturation plotted against cumulative degree-days was exponential in 1990 (Fig. 1a) and sigmoid in 1991 (Fig. 1b). A sigmoid curve was not obtained in 1990 because spore collections did not begin until after the pseudothecial population had entered the logarithmic phase of ascospore maturation. The number of degree-days required to reach 99% maturation of the season's ascospores was greater than predicted by the model (37), particularly in 1991. According to the model, approximately 520 degree-days were

required for 99% maturation of the season's ascospores, but 99% maturation was at 824 (1990) and 1125 (1991) degree days in the present study. Similar delays in maturation were noted when ascospore maturation was monitored with pseudothecial squash mounts prepared from 'McIntosh' leaves fully exposed to rain (Fig. 1a & 1b). Xeric conditions have been reported to inhibit ascospore maturation (59, 68, 79, 138), but moisture was not a limiting factor in this study because the leaf discs were wetted at weekly intervals, and leaves on the orchard floor were fully exposed the weather. Frequent exposure to high temperatures (> 25 C) may also have contributed to the delay in ascospore maturation. The model predicting ascospore maturity developed by Gadoury and MacHardy (37) was based on temperatures ≤ 20 C. Several studies have reported that ascospore maturation is slowed above 24 C, and inhibited above 28 C (59, 68, 138). Temperatures were above 25 C for 2 to 12 hours on 9 days in 1990 and 29 days in 1991 (Fig. 1). The Gadoury and MacHardy model was validated with field studies, but an analysis of weather records during their study show that the temperatures seldom exceeded 25 C. The results of the present study suggest that the model developed by Gadoury and MacHardy may overestimate the rate of ascospore maturation during days when the temperature exceeds 25 C for more than 2 hours.

Ascospore Productivity

Ascospore productivity was directly related to pseudothecial density, but with no apparent effect due to cultivar. In 1990, pseudothecial density was strongly correlated with RAP ($r^2 = 0.88$). The RAP and pseudothecial density was greatest on 'Rome,' 'Spartan,' and 'McIntosh' (Fig. 3). It had been reported (42) that pseudothecial density and ascal density were not influenced by cultivar, and it is unlikely that cultivar accounted for the differences in pseudothecial density recorded in 1990. Several studies have shown that pseudothecial density is strongly correlated with incidence of scab at the time of leaf-fall (22, 42, 138). In 1990, disease incidence was greatest on 'Rome' and 'McIntosh', and lowest on 'Mutsu' which had 88% less scab compared to 'McIntosh,' and this may explain

why the pseudothecial density on 'Mutsu' was significantly less than on 'Rome' or 'McIntosh' (Fig. 3b). Due to the heterothallic nature of *V. inaequalis*, an increase in pseudothecial density is likely to occur with an increase in disease incidence because the chance of compatible mating types occurring in close proximity on a leaf is increased. In 1991, the relative ascospore productivity, but not pseudothecial density differed significantly among the cultivars (Fig. 4). Relative ascospore productivity on 'Delicious' and 'Spartan' was relatively low, but this is believed due to the difficulty in accurately counting *V. inaequalis* spores because of an abundance of *Leptosphaeria sp. spores* on the slides (Fig. 5). *Leptosphaeria sp.* may have inhibited the production of pseudothecia through competition for nutrients or by antagonism (22, 51), and this could have accounted for the reduction in pseudothecial density in 'Mutsu' in 1990 (Fig. 3b).

Ascospore Maturation (squash mounts)

The rate of ascospore maturation determined from the squash mounts did not differ among the cultivars (Fig. 6), and this agrees with the spore trapping data. However, monitoring ascospore maturation by examining pseudothecial squash mounts is not as accurate as spore trapping data in predicting ascospore maturation (44).

Ascal Density (squash mount)

The maximum ascal density for each cultivar in 1990 was relatively low (45-85 asci/pseudothecium) compared to the 122 asci/pseudothecium reported by Gadoury and MacHardy (42). Temperatures between 15 Mar and 15 Apr 1990 averaged 4.1 C during the critical period for ascal development. This was the same average temperature reported by Gadoury et al. (43), so it was expected that the maximum ascal density would have been 122 (± 8) rather than the 45-85 observed in 1990. Only five squash mount assessments were made in 1990, so it is likely peak ascal density occurred between assessments and that the deterioration of empty asci before the next assessment could account for the low ascal density observed (39). The failure to accurately determine the maximum ascal density could account for the differences recorded in ascal density among the cultivars. If the

maximum ascal density had been determined, cultivar should have had little effect on ascal density, because temperature is the most important factor determining the ascal density (43, 42). The maximum ascospore productivity and the maximum ascal density both occurred between 0.635 cm (1/4") green tip and petal fall in all cultivars, a period lasting approximately three weeks. These results agree with several other studies (41, 49, 68, 87, 108, 128).

CHAPTER III

ANALYSIS OF SEVERAL COMPONENTS OF PARTIAL RESISTANCE THAT INFLUENCE APPLE SCAB BUILDUP

Introduction

Innate partial resistance to apple scab may be expressed as a reduction in the number of lesions per leaf or fruit, a reduction in lesion incidence on leaves and fruit, or as a reduction in pathogen growth and sporulation on the host. The components of partial resistance that influence the buildup of secondary scab could include the length of the incubation period, the number of conidia produced per lesion, and infectious period (the length of time a lesion produces conidia). Cultivar traits that contribute to avoidance (i.e. escape from infection) include the amount of susceptible tissue exposed at different host development stages and the time and rate at which the development stages occur in relation to the rate of ascospore maturation. These traits are not expressions of genetic resistance, but they may affect the buildup of scab. A few studies have compared susceptible apple cultivars for scab resistance, but the differences in resistance to *V. inaequalis* were not quantified. In one study (5), researchers and cooperative extension workers throughout the United States were asked to rate apple cultivars for scab resistance based on field observations. In the northeast, 'McIntosh' was rated least resistant, while 'Delicious' was rated moderately resistant. In France (96), 24 cultivars were grouped into three categories of scab resistance based on researchers opinions: most resistant, moderately resistant, and least resistant. The ratings in both these studies were based on subjective data not on quantitative or qualitative measures of resistance.

Only three studies have compared cultivars for conidium productivity or infectious period. Szkolnik (120) investigated conidial productivity among 30 apple cultivars under greenhouse and orchard conditions. Conidium productivity ranged from 185,000 conidia/lesion on 'Speigold' to 20 conidia/lesion on 'Priscilla' in the greenhouse, and from

514,000/lesion to 0 conidia/lesion on 'Speigold' and 'Priscilla,' respectively in the field. In a study by Hickey et al.(52), 300, 155, 82, 53, and 50 x 10^3 conidia/cm^2 lesion were collected from foliar lesions on 'Rome Beauty,' 'Delicious,' 'Golden Delicious,' 'Stayman,' and 'Cortland,' respectively. Both studies employed destructive sampling methods. The use of a non-destructive sampling method would have allowed the relative conidium production to be determined on individual lesions throughout their infectious period, and would have allowed the infectious period itself to be measured. Nusbaum and Keitt (92) reported that the center of lesions on 'Yellow Transparent' became necrotic 19 days after inoculation with *V. inaequalis* conidia. The necrotic zone increased until conidium production ceased at 30 - 36 days after inoculation.

Ontogenic resistance, inherent in all apple cultivars, is generally regarded as increased resistance to *V. inaequalis* concomitant with an increase in leaf age. Several studies have investigated ontogenic resistance in scab-resistant cultivars (9, 48, 124), but few have compared ontogenic resistance among partially resistant cultivars. Differences in the number of leaves infected on shoots of three different cultivars were noted by Nusbaum and Keitt (92). The three youngest leaves on 'Yellow Transparent' shoots developed sporulating lesions nine days after inoculation, while only the two youngest leaves on 'Fameuse' shoots developed non-sporulating lesions 12 days after inoculation. In the greenhouse, Szkolnik (120) noted that the severity (lesions/cm^2 leaf tissue) of scab on all susceptible cultivars studied decreased with leaf age. The youngest, second, third, and fourth leaves averaged 7.1, 4.5, 2.4, and 1.1 lesions/cm^2 leaf, respectively. Although these studies provide important information on ontogenic resistance among apple cultivars, there is still a lack of information comparing ontogenic resistance in cultivars under field conditions.

The objective of the field studies in this dissertation were to; (i) quantify the resistance to *V. inaequalis* in nine apple cultivars, (ii) quantify the susceptible tissue exposed at various phenological stages of fruit bud development, (iii) determine if the timing of the phenological stages differed, (iv) determine the incubation period, (v) quantify conidium

production/lesion, (vi) determine infectious period/lesion, and (vii) quantify ontogenic resistance, in nine apple cultivars.

Materials and Methods

Disease Assessments

All studies on disease development were conducted at the Woodman Horticultural Research Farm in Durham, New Hampshire. The orchard was a mixed planting of semi-dwarf apple trees on M-26 rootstock established in 1972. The trees were not treated with fungicides during the study, and insecticides were applied as necessary to keep insect damage at a minimum. Disease development in 1987 was insufficient for comparative studies. To insure sufficient scab development in following years, the orchard was "seeded" in the fall of 1987 with severely infected 'McIntosh' leaves collected from the Mast Road orchard in Durham.

Disease incidence (% infected leaves; % infected fruit) and severity (lesions/infected leaf; lesions/infected fruit) were assessed throughout the growing season by examining the leaves on 20 extension shoots and 50 fruit on each of three trees for each of nine cultivars. The leaves on 20 fruiting clusters were also assessed for scab development early in the growing season. The extension shoots, fruiting clusters, and fruit were randomly selected at each assessment date. The mean number of leaves/extension shoot was measured for each cultivar on each assessment date by counting the number of leaves/shoot on 20 randomly selected shoots. Disease assessments were made on 1 Jun, 12 Jun, 24 Jun, 7 Jul, 30 Jul, 16 Aug, and 12 Oct in 1988. In 1989, disease assessments were made at weekly intervals from 7 Jun to 15 Sep and on 3 Oct. In 1990, assessments were made every 14 days from 5 May to 14 Sep.

The tissues exposed at the green-tip stage of fruit bud development were marked with a felt-tip marker to determine which structures were the first to be exposed at this stage of bud development. Ten buds were marked in each cultivar. The marked spurs were then examined at the tight-cluster or pink stage of fruit bud development to identify the structure marked at green-tip. The incidence of sepal infection was also determined in 1990. At the time of fruit-set, sepals on 100 fruit from each cultivar were examined for scab lesions.

Several environmental variables (rainfall, temperature, relative humidity, and leaf wetness) were monitored and recorded with a modified hygrothermograph throughout the growing season to determine the timing of apple scab infection periods (84).

The logit of disease incidence was regressed against time (dates of observation) to compare the rate of disease development among the cultivars. The slope coefficients (b) of the regression equations were then compared to determine if cultivar influenced the rate of disease development on the foliage and fruit in any of the three years of the study (see Appendix Table 1). Disease severity and angularly transformed disease incidence data were compared by a series of one-way analysis of variance to determine if cultivar influenced the incidence or severity of scab on the fruit at the time of harvest or on the foliage just prior to natural leaf fall.

Leaf Area and Phenology

Leaf Area. The area of exposed green tissue was measured for the green-tip, 0.64 cm (1/4"), and 1.27 cm (1/2") stages of fruit bud development. Exposed green tissue was removed from each fruit bud and measured. A total of 30 fruiting buds was sampled at each of the three development stages. Leaf area (cm^2) of the unfurled leaves on 30 extension shoots and 30 fruiting clusters was measured at the open-cluster stage of fruit bud development. For each extension shoot, leaf position and the length of the shoot were recorded. The length (parallel to the mid-vein) and width (perpendicular to the mid-vein at the widest part of the leaf) of each extension shoot leaf were also recorded. Areas were determined using a photoelectric leaf area meter (LI-COR model LI-3000, Lambda Instruments Corp., Lincoln, NE). The measurements for leaves on each cluster were grouped to obtain the total leaf area per fruit cluster.

The mean leaf area and mean area of exposed green tissue were measured for each cultivar and all measurements were compared by one-way analysis of variance to determine if the means differed among the nine cultivars. The total leaf area/shoot was regressed against shoot length and the number of leaves/shoot to determine if shoot length or the number of leaves/shoot could predict total leaf area/shoot. Leaf area, measured

photoelectrically was regressed against leaf width, length, and the product of leaf width and length to determine which of these variables was the best predictor of leaf area.

Phenology. The phenological development of the fruit was recorded in 1987, 1989, and 1990. Fifty fruit buds on each of three trees of each of the nine apple cultivars were selected at random on each assessment date. The assessment dates were 20 and 26 Apr, 7, 11, 16, 26 May, and 1 Jun in 1987; 1, 15, 22, 29 May, and 7 Jun in 1989; 24 Apr, 2, 9, 14, 22 May, and 1, and 12 Jun in 1990. Fruit development was assessed based on the sixteen phenophases in Table 1. The phenophase of each of 150 fruit buds was recorded for each cultivar on each assessment date. To determine if fruit bud development differed among the cultivars, the sixteen phenophases were grouped into six "phenology groups" (Table 1), and the G Statistic was used to compare the number of fruit buds in each of the six "phenophase groups" at each assessment date. The "phenology groups" were determined by grouping phenophases that frequently "overlapped" at a single assessment date.

The number of leaves/shoot was recorded on 20 extension shoots of each cultivar at weekly intervals from 24 May to 9 Sept. The date of terminal bud set (date when all 20 extension shoots sampled had set terminal bud) was also recorded for each cultivar. After terminal bud-set, the number of leaves/shoot were compared by one-way ANOVA to determine differences among the cultivars. The number of leaves/shoot was regressed against time (dates of weekly assessments), and the slope coefficients (b) were compared to determine if there were differences in the rate of shoot growth (number of leaves unfurled/week) among the cultivars.

Conidial Productivity.

Conidia were collected from scab lesions on leaves on 20 and 29 Jun, 7, 15, 20, 25, and 31 Jul, 5, 10, 16, 21, 25, and 29 Aug, and 5, 10, and 15 Sep in 1989. In 1990, collections were made on 28 Jun, 5, 13, 20, and 29 Jul, 3, 9, 17, 24, and 30 Aug, and 6 Sep. Leaves with a single, barely visible (young) scab lesion were selected from 'Delicious,' 'McIntosh,' 'Stayman,' 'Mutsu,' 'Golden Delicious,' 'Spartan,' and 'Rome'

trees. Lesions did not develop in sufficient numbers on 'Paula Red' or 'Ida Red' for sampling in either year. Five leaves on lateral shoots within the tree canopy were selected for each cultivar. Lateral shoots within the canopy were desired because the canopy offered some protection from rainfall and wind. After the first sample was taken each year, the leaf and lesion were protected by a small "poly-house." The "poly-house" consisted of clear 0.5 mm polyethylene fitted over two 15 cm (ht) hoops of 18 ga galvanized wire. The plastic was secured to the wire with clear packing tape (Appendix Fig. 8). The "poly-house" was open on both ends to allow for air circulation and to prevent heat build-up. The length of the "poly-house" varied depending upon the size of the sample leaf and the lateral shoot. The exposed ends of the wire hoops were wrapped around twigs and branches to secure the "poly-house" over the sample leaf.

A non-destructive method for sampling conidia (Ken Hickey, personal communication) allowed sampling of the conidia from the same lesion for several weeks. The apparatus is shown in Fig. 1. Plastic "chap stick" tubes (67 x 10 mm) were chilled and then filled with 8 ml of sterile 1.5 % water agar. The first few mm of solidified agar were removed with a razor to provide a smooth, flat surface for collecting conidia. The tubes were then capped and refrigerated until needed. To collect conidia from a lesion, one mm (approximately) of agar was advanced out of the tube and the flat surface of the agar was pressed evenly against the lesion surface, taking care to avoid any lateral movement. The tube was lifted off the lesion and a thin (~ 1 mm thick) disc of agar was removed with a razor and placed, conidium-side up, in a sterile 100 x 50 mm plastic petri dish. The procedure was repeated once on each lesion. A total of ten agar discs were collected for each cultivar on each sampling date. A cotton swab dipped in lactophenol was taped to the top of the petri dish to inhibit spore germination, and the plates were refrigerated until the conidia were counted.

The agar discs were examined microscopically at 100x, and the relative conidium productivity for each lesion on each cultivar was determined by counting all conidia on both discs collected from a lesion for all collection dates. The infectious period (i.e., the number

of days conidia were produced), was determined for each of the five lesions on each cultivar. The size (cm^2) of the lesion was determined after the last conidial collection. The lesion was cut from the leaf and the area (cm^2) of the lesion was measured with a photoelectric leaf area meter.

The mean conidium productivity, infectious period, and lesion size for each of seven cultivars were compared by one-way analysis of variance to determine if there were significant differences in lesion productivity, infectious period, or lesion size among the cultivars. The rate of conidium production was determined for each cultivar by plotting the mean cumulative % of the total season's conidia collected for each sampling date.

Latent Period and Ontogenic Resistance

Smith Block. Six apple cultivars in a 0.25 ha planting (Smith block) were inoculated with conidial suspensions of *Venturia inaequalis* in 1990 and 1991. The Smith block, established in 1987, included 25 trees of each cultivar. The cultivars were 'Redchief,' 'Lawspur', 'Mutsu,' 'Paula Red,' 'Macspur,' and 'Smoothee' on MM9/106, MM9/111, M-26, M-26, M-26, and MM9/106 rootstock, respectively. Benlate (benomyl) 50 WP was applied to the trees (34.4g/ha a.i.) in mid-Oct in 1989 and in 1990 to suppress pseudothecium formation (104, 40). The leaves were raked and removed from the block after leaf-fall each year and again prior to bud-burst the following spring. In 1990, fungicides were applied to the Smith block to prevent infections by *V. inaequalis* from a nearby source of inoculum (crabapple trees that were removed in Aug, 1990). In 1990, Fenarimol 1EC (0.86 L/ha a.i.) was applied on 8, 12, 19, and 23 Jun, and Mancozeb 80 WP (2.94 kg/ha a.i.) was applied on 12 and 19 Jun. In 1991, Dodine 65 WP (.71 kg/ha a. i..) and 80 WP (2.94 kg/ha a. i..) were applied on 10 May. Insecticides were applied as necessary to reduce insect damage.

The youngest eight leaves on ten extension shoots of each cultivar were inoculated in the Smith block on 6 and 12 Jul in 1990, and 18 Jun and 5 Jul in 1991. The petiole of the youngest unfurled leaf was marked with yellow tape, and all but the eight youngest leaves were removed from the shoots. The conidium inoculum was prepared by rinsing heavily

scabbed 'McIntosh' leaves in distilled water and adjusting the inoculum suspension to 50,000 conidia/ml in 1990 and 200,000 conidia/ml in 1991 with the aid of a hemocytometer. The conidial suspension was applied uniformly to the leaves until run-off with a hand-pump spray bottle in 1990. In 1991, the conidial suspension was applied uniformly to each leaf with an air brush (Model type H, Paasch Airbrush Co., Chicago, IL) to achieve a deposit of 180 to 240 conidia/cm^2 leaf. Microscope slides were sprayed with the conidial suspension after every three extension shoots were inoculated to determine the actual concentration of conidia being applied to the leaves. After the conidial suspension dried, the leaves were misted with distilled water and the shoots were covered with white plastic bags which had been misted with water on the interior. The open end of each bag was loosely secured with a twist-tie to maintain high humidity and to delay drying of the leaves. Control shoots were misted with water and covered with plastic bags but were not inoculated. The bags were left in place for 18 - 29 hr, depending on the environmental conditions. In 1991, petri dishes (100 x 15 mm) filled with 10 ml of sterile 15% water agar were sprayed with the conidial suspension and placed, covered and uncovered, in misted plastic bags within the canopies of the inoculated trees or covered on the orchard floor. Assessments of disease incidence and severity were made at 3-day intervals beginning six days after inoculation and continuing until a lesion first appeared on any leaf that had unfurled after inoculation.

The incubation period (days from inoculation to the first appearance of sporulating lesions) was determined for shoots (IPS = days to first lesion/extension shoot for 10 shoots) and leaves (IPL = days to first lesion/leaf for all infected leaves). The data were analyzed by a series of one-way analysis of variance to determine if there was a significant cultivar effect on scab severity, IPS, and IPL. The number of lesions/infected leaf was regressed against IPL to determine if scab severity was correlated with incubation period. Linear regression analysis was used to determine the correlation between leaf position on a shoot and scab severity and IPL.

Potted Trees. Nine apple cultivars in 6 inch plastic pots were inoculated with conidium suspensions on 11 Jun in 1990 and 30 Jun in 1991. The potted trees, grafted on M-26 rootstock in 1989, included 3-5 trees of each cultivar. The cultivars were 'Delicious,' 'McIntosh,' 'Stayman,' 'Mutsu,' 'Paula Red,' 'Ida Red,' 'Golden Delicious,' 'Spartan,' and 'Rome.' The potted trees did not receive fungicide applications. Insecticides were applied as necessary to reduce insect damage. The petiole of the youngest unfurled leaf on each extension shoot of the potted trees was marked with yellow tape beginning 19 May in 1990 and 25 May in 1991. As each additional shoot began to expand, the youngest leaf was marked and the shoot was included in subsequent leaf-age assessments. The number of unfurled leaves was recorded daily to determine the age of each leaf at the time of inoculation. The youngest unfurled leaf was marked with blue tape at the time of inoculation. The conidial suspension (50,000/ml) was applied uniformly to the leaves with air brush until run-off in 1990. In 1991, the conidial suspension (100,000/ml) was applied uniformly with an air brush to achieve a final deposit of 90 to 120 conidia/cm^2 leaf surface. After the spore suspension dried for 30 min, the potted trees were placed in a mist chamber at 15.5 to 17.8 C and 100% relative humidity for 36 hr. The potted trees were then removed from the chamber and placed outdoors in a location several miles from any known source of apple scab inoculum. Beginning six days after inoculation in 1990, the trees were assessed daily for scab lesion incidence and severity. In 1991, disease assessments were made at three-day intervals beginning when the first lesion was observed. Disease assessments continued until a lesion appeared on a leaf that had unfurled after inoculation.

The incubation period was determined for shoots (IPS = days to first lesion/extension shoot) and leaves (IPL = days to first lesion/leaf for all infected leaves). The data were analyzed by a series of one-way analysis to determine if cultivar had a significant effect on lesion severity, IPS, IPL, and the age of infected leaves when inoculated. The number of lesions/infected leaf was regressed against IPL to determine if lesion severity was correlated with latent period. Linear regression analysis was used to determine the

correlation between leaf age of infected leaves at the time of inoculation with lesion severity and IPL.

Table 1. Phenophases (growth stages) of apple fruit bud development and corresponding "phenophase group" used in statistical analysis.

Phenophase	Fruit development stage	Phenology group
1	Dormant	1 (1-3)
2	Silver-tip (ST)	
3	Green-tip (GT)	
4	0.64 cm GT	2 (4 + 5)
5	1.27 cm GT	
6	1-2 cluster leaves expanded	3 (6-10)
7	3-5 cluster leaves expanded	
8	Tight-cluster (TC)	
9	1 floral bud away from TC	
10	2-3 floral buds away from TC	
11	Open-cluster (OC)	4 (11 + 12)
12	Pink	
13	Bloom	5 (13)
14	1-2 flowers' petals fallen	6 (14-16)
15	Petal-fall	
16	Fruit-set	

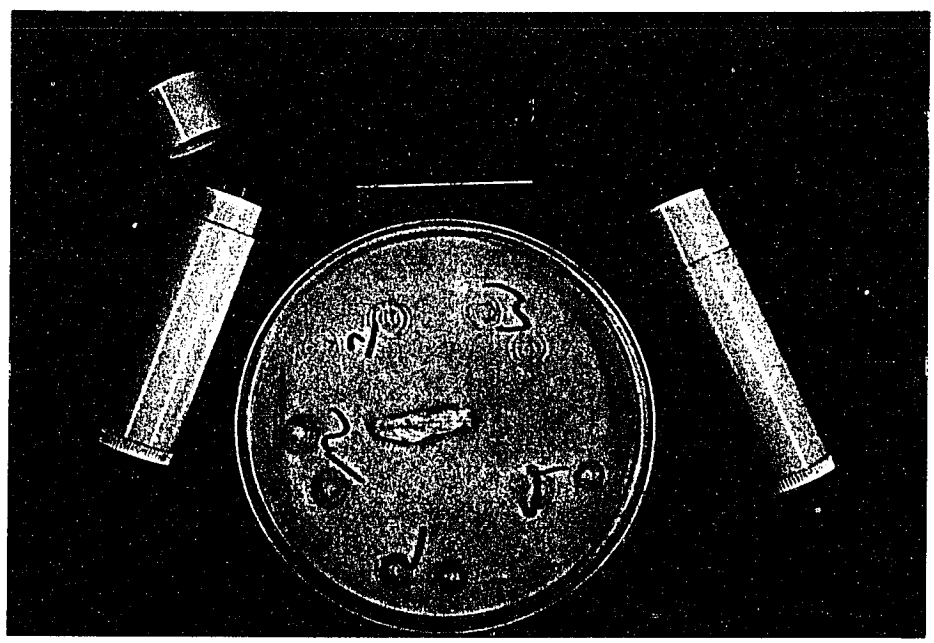


Fig. 8. Apparatus for non-destructive sampling of conidia of *Venturia inaequalis* on scab lesions. Shown in the photo are two "chap-stick" tubes, one uncapped to show the exposed agar; a razor blade for slicing the agar; and a petri dish with agar discs and lactophenol-soaked cotton.

Results

Disease Assessments

Disease Development on Extension Shoot Leaves. The rate of disease development on extension shoot leaves differed significantly among the cultivars. Three general patterns of disease development were observed in all three years of the study: (i) disease incidence increased throughout the growing season, (ii) an initial increase of 3-5% in disease incidence with no further increase in incidence during the remainder of the growing season, and (iii) either no disease or an initial increase of 1-3% in disease incidence with no increase in disease for the remainder of the growing season.

In 1988, the rate of disease development was significantly ($P = 0.05$) greater on 'Stayman' and 'Rome' than on any other cultivar (Fig. 9). The rate of disease development on 'McIntosh,' 'Mutsu,' and 'Delicious' was greater than the rate on 'Spartan' and 'Golden Delicious.' Disease incidence on 'Ida Red' increased 7% during Jun followed by a decrease of 6% in Jul. There was no disease development on 'Paula Red.' In 1989, the incidence of leaf scab increased at a significantly ($P = 0.05$) higher rate on 'Rome' and 'McIntosh' compared to the other seven cultivars (Fig. 10). There was no scab development on 'Paula Red.' In 1990, 'McIntosh' and 'Rome' had significantly ($P = 0.05$) higher rates of disease development compared to the other cultivars (Fig. 11). Disease incidence increased approximately 10% on 'Spartan' during the latter part of Jun, but, there was no further increase in disease, and, in fact, disease incidence slowly decreased during the remainder of the growing season. No scab lesions developed on 'Ida Red.' A slight increase (0.1%) in disease incidence on 'Delicious' and 'Paula Red' was the result of a single infected leaf.

At leaf-fall, the incidence and severity of scab on extension shoot leaves differed significantly among the cultivars. In 1988, lesion incidence ranged from 79% ('Stayman') to 0% ('Paula Red') and was significantly ($P = 0.05$) greater on 'Stayman' leaves compared to the other cultivars (Fig. 12a) Lesion incidence on 'McIntosh' and 'Rome'

was significantly (P = 0.05) greater than on the other six cultivars. The reduction in lesion incidence on the other cultivars relative to 'Stayman' was 55 (MC), 56 (RO), 82 (MU), 89 (SP), 94 (RD) and (IR), 99 (GD), and 100% (PR). Lesion severity ranged from 7.9 lesions/leaf on 'Stayman' to 0.0 lesions/leaf on 'Paula Red' (Fig 12b). Lesion severity at the time of leaf-fall was significantly (P = 0.05) greater on 'Stayman' compared to 'Rome,' and the severity on both cultivars was significantly greater than on any of the other cultivars. Between the end of the primary season and the end of the secondary season, lesion severity increased on four cultivars (ST, RO, MU, and RD) and decreased on four cultivars (MC, IR, SP, and GD).

In 1989, the incidence of lesion on extension shoot leaves ranged from 48% on 'McIntosh' to 0% on 'Paula Red' and 'Ida Red,' and was significantly (P = 0.05) greater on 'McIntosh' compared to the other cultivars (Fig. 13a). The reduction in lesion incidence on the other cultivars relative to 'McIntosh' was 52 (RO), 78 (ST), 83 (GD and SP), 88 (MU), 94 (RD), and 100% (PR and IR). Lesion severity at leaf-fall ranged from 5.9 lesions/leaf on 'Rome' to 0.0 lesions/leaf on 'Paula Red' and 'Ida Red,' and was significantly (P = 0.05) greater on 'Rome,' 'Mutsu,' and 'McIntosh' than on 'Delicious,' 'Paula Red,' and 'Ida Red' (Fig 13b). Between the beginning and the end of the secondary scab season, scab severity increased on all cultivars except 'Paula Red' and 'Ida Red' .

In 1990, the incidence of lesion on extension shoot leaves ranged from 38% on 'McIntosh' to 0.0% on 'Ida Red,' and was significantly (P = 0.05) greater on 'McIntosh' and 'Rome' compared to the remaining seven cultivars (Fig. 14a). A single infected leaf accounted for the 0.1% scab incidence on 'Paula Red' and 'Delicious.' The reduction in lesion incidence on the other cultivars relative to 'McIntosh' was 30 (RO), 89 (MU and SP), 94 (GD), 97 (ST), 99.9 (PR and RD), and 100% (IR). Scab severity ranged from 5.3 lesions/leaf (RO) to 0.0 lesions/leaf (IR). Between the beginning and end of the secondary scab season, lesion severity increased on all cultivars except 'Ida Red' which was scab-free in 1990 (Fig. 14b).

Disease Development on Fruit. The rate of increase in the incidence of scab on the fruit differed significantly (P = 0.05) among the cultivars in 1989 and 1990. In 1988, the rate of disease development on the fruit did not differ among the cultivars although there was a significant increase in the incidence of fruit scab on both 'McIntosh' and 'Rome' between 16 Aug and 12 Oct (Fig. 15). In 1989, the rate of increase in disease development was significantly greater on 'McIntosh' and 'Rome' compared to the other cultivars (Fig. 16). The incidence of scabbed fruit remained < 7% throughout the growing season except on 'Spartan' where the incidence increased to 12% by the final assessment on 24 Aug. In 1990, disease developed at a significantly (P = 0.05) higher rate on 'McIntosh' and 'Rome' fruit compared to the other cultivars (Fig. 17). The incidence of scabbed fruit on 'Spartan' was greater than on any other cultivar throughout 1990.

At the final assessment for fruit scab, the incidence and severity of fruit scab differed significantly among the cultivars. In 1988, the incidence of scabbed fruit ranged from a 70% on 'McIntosh' to 2% on 'Golden Delicious,' and significantly more 'McIntosh' fruit were diseased compared to the other cultivars (Fig. 18a). The incidence of scabbed fruit was 47, 51, 60, 73, 80, 88, 95, and 97% less on 'Rome,' 'Stayman,' 'Delicious,' 'Ida Red,' 'Mutsu,' 'Spartan,' 'Paula Red,' and 'Golden Delicious' relative to 'McIntosh,' respectively. The severity of fruit scab ranged from 9.6 lesions/infected fruit (MC) to 1.7 lesions/infected fruit (PR), and was significantly greater on 'McIntosh' and 'Rome' compared to the other cultivars (Fig 18b). In 1989, the incidence of scabbed fruit ranged from 48% (MC) to 0% (PR), and was significantly (P = 0.05) greater in 'McIntosh' compared to the other eight cultivars (Fig. 19a). The incidence of scabbed fruit was reduced 64, 74 85, 86, 90, 94, 97, and 100% on 'Rome,' 'Spartan,' 'Stayman,' 'Golden Delicious,' Mutsu,' 'Delicious,' 'Ida Red,' and 'Paula Red' relative to 'McIntosh,' respectively. The severity of scabbed fruit ranged from 9.3 lesions/infected fruit (RO) to 0.0 lesions/infected fruit (PR) (Fig 19b). In 1990, the incidence of scabbed fruit ranged from a 62% (SP) to 0.0% (PR), and 'Spartan' had a significantly (P = 0.05) greater number of infected fruit compared to the other eight cultivars (Fig. 20a). The incidence of

scabbed fruit was reduced 37, 38, 60, 81, 86, 90, 98, and 100% on 'McIntosh,' 'Rome,' 'Mutsu,' 'Delicious,' 'Stayman,' 'Golden Delicious,' 'Ida Red,' and 'Paula Red' relative to 'Spartan,' respectively. The severity of fruit scab ranged from 7 lesions/infected fruit on 'Stayman' to 0.0 lesions/fruit on 'Paula Red' (Fig. 20b). Scab lesions on 'Spartan' fruit were very large, often covering approximately 50% of the fruit (Fig 21).

Disease Development on Sepals and Spur Leaves. The incidence of scab lesions on sepals ranged from 19% on 'Spartan' to 0.0% on 'Ida Red' (Fig. 22). Buds marked at the green-tip stage and examined at tight-cluster demonstrated that sepals were the only green tissue exposed at the green tip stage of bud development in 80% to 100% of the buds on all cultivars. In the remaining buds, the tips of spur leaves were exposed at green-tip.

The incidence and severity of scab on spur leaves differed significantly among the cultivars (Figs. 23-25). Spur leaves on 'Paula Red' did not develop scab lesions in the three years they were assessed, and lesions did not occur on the spur leaves on 'Mutsu' and 'Ida Red' in 1989 (Fig. 24). The incidence of scab lesions on spur leaves in 1988 was 4, 7, 44, 64, 84, 93, and 100% on 'Mutsu,' 'Rome,' 'McIntosh,' 'Spartan,' 'Delicious,' 'Golden Delicious,' and 'Paula Red' relative to 'Stayman' and 'Ida Red,' respectively (Fig. 23a). The severity of scab was greatest on 'Rome' (2.9 lesions/infected leaf) (Fig. 23b). In 1989, the reduction in spur leaf incidence on the other cultivars relative to 'Rome' was 29 (MC), 55 (RD), 77 (GD), 90 (SP and ST), and 100% (MU, PR, and IR).(Fig. 24a). Scab severity was greatest on 'McIntosh': infected leaves averaged 2.2 lesions (Fig. 24b). In 1990, the incidence of scab on spur leaves was 55, 58, 76, 91, 93, 95, and 100% on 'Mutsu,' 'Rome,' 'Golden Delicious,' 'Stayman,' 'Ida Red,' 'McIntosh,' and 'Paula Red' relative to 'Spartan,' respectively (Fig. 25a). Scab severity ranged from 2.2 lesions/infected leaf on 'Stayman' to 0.0 lesions/leaf on 'Paula Red' (Fig. 25b).

Leaf Area and Phenology

Leaf area. The area of exposed green tissue differed significantly ($P = 0.05$) among cultivars at all four stages of fruit bud development. Differences among the cultivars were most pronounced at the open-cluster stage of development (Fig. 26). The area of exposed

tissue (total area of all cluster leaves) was significantly greater on 'Rome' than on the other eight cultivars, and ranged from 70 cm^2 on 'Rome' to 14 cm^2 on 'McIntosh.' At the green-tip stage of bud development, the nine cultivars were separated into three groups based on the statistical analysis (Fig. 27a): 0.48 - 0.42 cm^2 (IR, MU, PR), 0.32 - 0.27 cm^2 (GD, ST, MC), and 0.19 - 0.11 cm^2 (RD, SP, RO). The area of exposed tissue did not differ significantly among the cultivars within a group, but it did differ when the cultivars were compared between the groups.

The area of tissue exposed at the 0.64 cm (1/4") green-tip stage of development was significantly (P = 0.05) greater on 'Ida Red' compared to the other cultivars, and ranged from 1.5 cm^2 on 'Ida Red' to 0.06 cm^2 on 'McIntosh' (Fig. 27b). At 1.27 cm (1/2") green-tip, the differences in area of tissue exposed were less apparent. The amount of tissue exposed on 'Delicious' buds was significantly (P = 0.05) less compared with the other eight cultivars (Fig. 27c). The 0.64 cm green- and 1.27 cm green-tip stages of bud development usually "overlapped" in the field (see Figs. 28-30), so the measurements for both bud stages were combined and analyzed (Fig. 28). A greater amount of tissue was exposed on 'Ida Red' buds (4.1 cm^2) compared to the other cultivars, particularly to 'Delicious' which had only 2.4 cm^2 of exposed tissue.

The leaf area of extension shoot leaves differed significantly (P = 0.05) among the cultivars. The areas of the five youngest unfurled leaves of each cultivar are shown in Table 2. The third, fourth, and fifth leaves on 'Mutsu' shoots were larger than similarly positioned leaves of the other cultivars, while the leaves of 'Delicious' were consistently smaller than those of any other cultivar. The area of leaf number 1, (the youngest leaf), ranged from 5.8 cm^2 on 'Spartan' to 2.0 cm^2 on 'Delicious,' and was significantly (P = 0.05) greater on 'Spartan' compared to the other cultivars. Leaf number 2 was significantly larger on 'Spartan,' than leaf number 2 in the other cultivars except 'Mutsu' and 'Rome.' When the area of leaves 1-5 were combined, 'Mutsu' exceeded the other cultivars by 24% (RO), 26% (SP), 36% (GD), 40% (PR), 41% (ST), 57% (MC), 70% (IR), and 71% (RD).

The relationship between leaf area and leaf width, leaf length, the product of leaf width and length, leaves/shoot, and extension shoot length was determined by regression analysis and the results are shown in Table 3. The best predictor of leaf area was the product of leaf width and leaf length ($R^2 \geq 0.98$ for all cultivars). The R^2 for leaf width as the predictor of leaf area was ≥ 0.90 for all cultivars but 'Delicious,' ($R^2 = 0.85$). The R^2 value was 0.87 for both 'Delicious' and 'Paula Red,' and ≥ 0.90 for the remaining seven cultivars when leaf length was regressed against leaf area. Since leaf area and the product of leaf length and width was strongly correlated for each cultivar, a leaf area index (LAI) number was calculated for each cultivar. The LAI was equal to the quotient of the actual leaf area (measured photoelectrically) divided by the product of leaf length and leaf width. The LAI was 0.74 (RD), 0.76 (MC), 0.71 (ST), 0.69 (MU), 0.76 (PR), 0.74 (IR), 0.70 (GD), 0.74 (SP), and 0.70 (RO). The predicted leaf area obtained when LAI was multiplied by the product of leaf length and width varied from the actual leaf area by less than 0.5 %.(.08 cm^2).

The length of an extension shoot was a very poor predictor of the total leaf area/shoot ($R^2 \leq 0.49$ in all cultivars). The number of leaves/shoot was also a poor predictor of the total leaf area/shoot. Only 'Mutsu' and 'Golden Delicious' had R^2 values greater than 0.70 (0.74 and 0.71, respectively), and R^2 values for the other cultivars was < 0.70.

Phenology. The rate of development of the fruit buds differed significantly ($P = 0.01$) among the cultivars on each assessment date in 1987, 1989, and 1990. The development of fruit buds based on 16 phenophases (see Table 1) is shown in Figs. 29-31, and the development based on "phenology groups" is shown in Appendix Figs. 5-7. In 1987, the fruit buds on 'Ida Red' and 'McIntosh' developed more rapidly than on the other cultivars (Fig. 29 and Appendix Fig. 5). The development of the fruit buds in 'Paula Red' was similar to 'Ida Red' and 'McIntosh' except on 26 May when development on 'Paula Red' lagged behind 'Ida Red' and 'McIntosh.' Fruit bud development lagged on 'Rome' compared with the other cultivars on all assessment dates except 20 Apr. On 20 Apr, fewer buds on 'Golden Delicious' and 'Spartan' had broken dormancy and reached the 0.64 cm

(1/4") green-tip stage of development. The ranking of the cultivars from fastest to slowest fruit bud development in 1987 was 'Ida Red,' 'McIntosh,' 'Paula Red,' 'Mutsu,' 'Stayman,' 'Spartan,' 'Golden Delicious,' and 'Rome.'

In 1989, the most rapid fruit bud development occurred on 'Ida Red' and 'McIntosh' compared to the other cultivars (Fig. 30 and Appendix Fig. 6). Although the buds on 'Rome' broke dormancy at the same time as the other cultivars, development of the buds lagged. By 29 May there were few differences in the stage of development of the fruit buds among the cultivars except on 'Paula Red' and 'Rome' which still had 15% and 16% of the buds at bloom, respectively, compared to less than 3% on 'Golden Delicious' and ≤ 1% on the remaining cultivars. On 7 May, all cultivars were at the fruit-set stage. The ranking of the cultivars from fastest to slowest fruit bud development in 1989 was 'Ida Red,' 'McIntosh,' 'Spartan,' 'Mutsu,' 'Delicious,' 'Golden Delicious,' 'Spartan,' 'Paula Red,' and 'Rome.'

In 1990, fruit buds were most advanced on 'Ida Red'at all assessment dates except 24 Apr (Fig. 31 and Appendix Fig. 7). On 24 Apr, all fruit buds on 'Golden Delicious' and 'Rome' were in "phenology group" 2 (Appendix Fig. 7) compared to 65% on 'McIntosh' and ≤ 42% in the other cultivars. Although delayed when compared to 'Ida Red,' the development of the fruit buds on 'McIntosh' and 'Paula Red' was more advanced than the other cultivars on 9 May and 14 May. On 9 May, 25% and 10% of the fruit buds on 'McIntosh' and 'Paula Red,' respectively, were in "phenology group" 4 (Appendix Fig. 7) compared to less than 1% on the other cultivars. On 14 May, 59% and 42% of the fruit buds on 'Paula Red' and 'McIntosh,' respectively, were in "Phenology group" 5 compared to ≤ 25% on the other cultivars. On 12 Jun, all cultivars were at petal-fall (Fig. 31). The ranking of the cultivars from fastest to slowest fruit bud development was 'Ida Red,' 'McIntosh,' 'Paula Red,' 'Mutsu,' 'Spartan,' Stayman,' 'Golden Delicious,' 'Delicious,' and 'Rome.'

There were no significant (P = 0.05) differences in the maximum number of leaves/shoot or the rate of shoot growth (no. leaves unfurled/week) among the nine

cultivars (Table 4, and Fig. 32). The number of leaves/shoot ranged from 25 (MC) to 21 (MU, PR, AND GD). The rate of shoot growth ranged from 1.9 leaves unfurled/week on 'McIntosh' to 1.3 leaves unfurled/week on 'Paula Red.' On 20 Jul, 100% of the extension shoots examined on 'Paula Red' had set terminal buds. All cultivars reached 100% terminal bud-set by 31 Aug.

Conidium Productivity.

The relative conidium productivity differed significantly (P = 0.05) among the seven cultivars in 1989 and 1990. In 1989 (Fig. 33a), conidium productivity was significantly greater on 'Golden Delicious,' 'Mutsu,' 'Rome,' and 'Stayman' lesions compared to 'McIntosh,' 'Spartan,' and 'Delicious' lesions. Productivity ranged from approximately 15,000 to 29,534 conidia/lesion in the first group and from 5,379 to 9,732 conidia/lesion in the second group. In 1990 (Fig. 33b), productivity was significantly greater on 'Golden Delicious,' 'Rome,' and 'Spartan' lesions compared to lesions on the other four cultivars, and ranged from 15,342 to 30,719 conidia/lesion in the first group to 3,507 to conidia/lesion on 'Delicious' leaves. The area of scab lesions in 1989 ranged from 0.4 cm^2 on 'Mutsu' to 0.25 cm^2 on 'Red Delicious leaves, but there was no significant difference in lesion size among the seven cultivars (Fig. 34).

The length of time lesions remained infectious (infectious period), i.e., produced conidia, differed significantly among the cultivars in 1989 and in 1990. In 1989, the mean infectious period varied from 94 days on 'Golden Delicious' lesions to 51 days on 'McIntosh' and 'Spartan' lesions, and was significantly longer (P = 0.05) on 'Golden Delicious' lesions than on lesions of the other six cultivars (Fig. 35a). In 1990, the mean infectious period varied from 68 days on 'Golden Delicious' lesions to 52 days on 'McIntosh' lesions, and was significantly longer (P = 0.01) for lesions on 'Golden Delicious,' 'Stayman,' 'Rome,' and 'Mutsu' compared with lesions on 'Delicious,' 'Spartan,' and 'McIntosh' (Fig. 35b). On the first day of sampling (day 0) in 1989, the percentage of the season's conidium production was significantly greater (P = 0.05) on 'Spartan' and 'Rome' lesions compared with lesions on 'McIntosh' and 'Rome,' only

(Fig. 36a). On day 9, the percentage of the season's conidium production was significantly less (P = 0.05) on 'Delicious' lesions than on lesions on any other cultivar. In 1990, the percentage of the season's conidium production on day 0 was significantly greater (P = 0.05) on 'Rome' and 'Spartan' lesions compared to the conidium production on lesions on all other cultivars (Fig. 36b). By day 7, lesions on 'Rome' had produced a greater percentage of the season's conidium production compared to lesions on 'Stayman,' 'Delicious,' and 'McIntosh.'

The number of days required to reach 90 % of the season's conidium production ranged from 23 days on 'Stayman' lesions to 28 days on 'Red Delicious lesions in 1989, and from 23 days on 'Rome' lesions to 32 days on 'Delicious' lesions in 1990 (Table 5). The lesions remained infectious longer in 1990 compared to 1989. The correlation between the infectious period and total conidium production was strong in 1989 ($R^2 = 0.76$), but very weak in 1990 ($R^2 = 0.31$).

Ontogenic Resistance and Latent Period

Smith Block

Disease assessments. Scab severity differed significantly (P = 0.10) among the cultivars inoculated on 12 Jul, 1990 and 5 Jul 1991. Apple scab lesions did not develop on any cultivar inoculated on 6 Jul 1990 or 18 Jun 1991. Too few scab lesions developed on leaf #3 or older leaves for comparisons by statistical analysis in either 1990 or in 1991. In 1990, scab severity on leaf #1 (the youngest unfurled leaf at the time of inoculation) ranged from 12 lesions/leaf on 'Mutsu' to 2 lesions/leaf on 'Paula Red' (Fig. 37a), and was significantly greater on 'Mutsu' than on all other cultivars except 'Delicious' (Table 6). The severity of scab on leaf #2 ranged from 6 lesions/leaf on 'Delicious' to 0 lesions on 'McIntosh' and 'Paula Red' (Fig. 37a), but did not differ significantly among the cultivars (Table 6). The incidence of scab on leaf #1 ranged from 80 % on 'Mutsu,' 'Delicious,' and 'Rome' to 20 % on 'Paula Red,' and the incidence on leaf #2 ranged from 60 % on 'Mutsu' to 0 % on 'Paula Red' (Fig. 37b). In 1991, scab severity on leaf #1 was

significantly less on 'Golden Delicious' and 'Paula Red' compared to the other cultivars (Table 6), and ranged from 42 lesions/leaf on 'Delicious' to 0 lesions/leaf on 'Paula Red' (Fig. 38a). The severity of scab on leaf #2 ranged from 27 lesions/leaf on 'McIntosh' to 0 lesions/leaf on 'Paula Red.' The incidence of scab on leaf #1 ranged from 90 % on 'McIntosh' to 0 % on 'Paula Red,' and on leaf #2 from 27 % on 'McIntosh' to 0 % on 'Paula Red' (Fig. 38b).

Incubation period. The mean incubation period for shoots (IPS) did not differ significantly among the cultivars in either 1990 or 1991 (Table 6). The mean incubation period for infected leaves (IPL) did not differ significantly among the cultivars in 1990 or 1991 (Table 6). The incubation period ranged from 16 days on 'McIntosh' and 'Delicious' leaves to 23 days on 'Paula Red' leaves in 1990 (Fig. 39a), and from 14 days on 'Mutsu' leaves to 17 days on 'Rome' in 1991(Fig. 39b).

Potted trees

Scab severity and incidence. The severity of scab lesions on the youngest two leaves did not differ among the cultivars in 1990 or 1991 (Table 7). In 1990, the scab severity on leaf #1 ranged from 16 lesions/leaf on 'Stayman' to 0 lesions/leaf on 'Paula Red' (Fig. 40a). The severity on leaf #2 ranged from 16 lesions/leaf on 'McIntosh' to 0 lesions/leaf on 'Paula Red. The incidence of scab on leaf #1 ranged from 100 % on 'Golden Delicious' to 0 % on 'Paula Red,' and on leaf #2 ranged from 100 % on 'Stayman' to 0 % on 'Paula Red' (Fig. 40b). In 1991, scab severity on leaf #1 ranged from 8 lesions/leaf on 'Spartan' to 0 lesions/leaf on 'Paula Red' (Fig. 41a). Scab severity on leaf #2 ranged from 12 lesions/leaf on 'Rome' to 0 lesions/leaf on 'Paula Red.' The incidence of scab on leaf #1 ranged from 54 % on 'McIntosh' to 20 % on 'Stayman,' and on leaf #2 from 55 % on 'McIntosh' to 0 % on 'Paula Red' (Fig. 41b).

Incubation period. The incubation period differed significantly among the cultivars in 1990 but not in 1991. In 1990, the IPL was significantly longer on 'Mutsu' compared to all cultivars except 'Spartan' and 'Rome' (Table 7), and ranged from 11 days on 'Stayman' to 16 days on 'Mutsu' (Fig. 42a). The IPS differed significantly among the cultivars in

1991 but not in 1990 (Table 7). In 1991, scab lesions were visible on 'Rome' and 'Paula Red' extension shoots 11 days after inoculation, but lesions were not visible on 'Golden Delicious' extension shoots until 26 days after inoculation (Fig. 42b).

Leaf age. At the time of inoculation, leaves on 'Delicious,' 'McIntosh,' and 'Rome' were significantly ($P = 0.10$) younger than the leaves on 'Spartan' and 'Mutsu' in 1990, and leaves on 'Stayman,' 'Golden Delicious,' 'Spartan,' and 'Ida Red' in 1991. The mean age of the leaves at the time of inoculation in 1990 was 16 (MU), 13 (SP), 13 (PR), 12 (IR), 12 (GD), 11 (ST), 10 (MC), 8 (RO), and 8 (RD) days. The mean age of the leaves at the time of inoculation in 1991 was 25 (ST), 25 (GD), 24 (SP), 23 (IR), 22 (MU), 21 (PR), 20 (RD), 19 (MC), and 18 (RO) days.

The mean age of all leaves (at the time of inoculation) that developed lesions differed significantly ($P = 0.05$) among the cultivars in 1990, but not in 1991 (Fig. 43). However, when leaves at position #1 or #2 only were compared, there were no differences in the age of scabbed leaves at the time of inoculation among the cultivars. In 1990, the mean age of #1 leaves that developed scab lesions was 14 (MU), 11 (SP), 8 (GD), 8 (IR), 6 (ST), 1 (MC), 1 (RO), and 1 (RD) days old at the time of inoculation. The mean age of #2 leaves that developed scab lesions was 15 (MU), 12 (SP), 10 (GD), 10 (ST), 8 (IR), 7 (MC), 6 (RD), and 6 (RO) days old at the time of inoculation . In 1991, the mean age of the #1 leaves that developed scab lesions was 25 (ST), 16 (RD), 15 (IR), 14 (MC), 12 (MU), 10 (GD), 7 (SP), 4 (PR), and 3 (RO) days old at the time of inoculation. In 1990, 58% of the leaves younger than 13 days at inoculation developed scab compared to 21 % of the leaves older than 13 days. In 1991, 63 % of the leaves younger than 13 days at inoculation developed scab compared to 10 % of the leaves older than 13 days.

Regression analysis. There was a weak ($R^2 = 0.49$) correlation between leaf position on a shoot and IPL in the Smith block in 1991 (Table 8). The incubation period increased approximately 7 days with each successively older leaf on a shoot. A weak correlation ($R^2 = 0.30$) existed between IPL and the severity of apple scab infections in the potted trees.

The number of lesions/infected leaf decreased by 1.72 lesions for each day the incubation period increased. The remaining regressions between leaf position and severity, leaf age and severity, and leaf age and IPL were all poorly correlated ($R^2 < 0.30$).

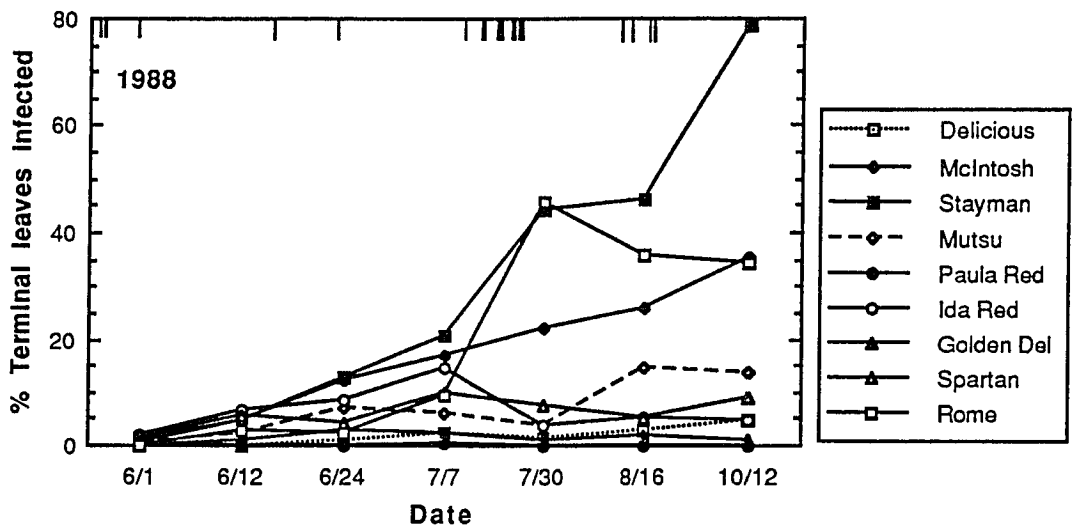


Fig. 9. Incidence of apple scab caused by *Venturia inaequalis* on extension shoot leaves during 1988. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch mark indicates the duration of the infection period.

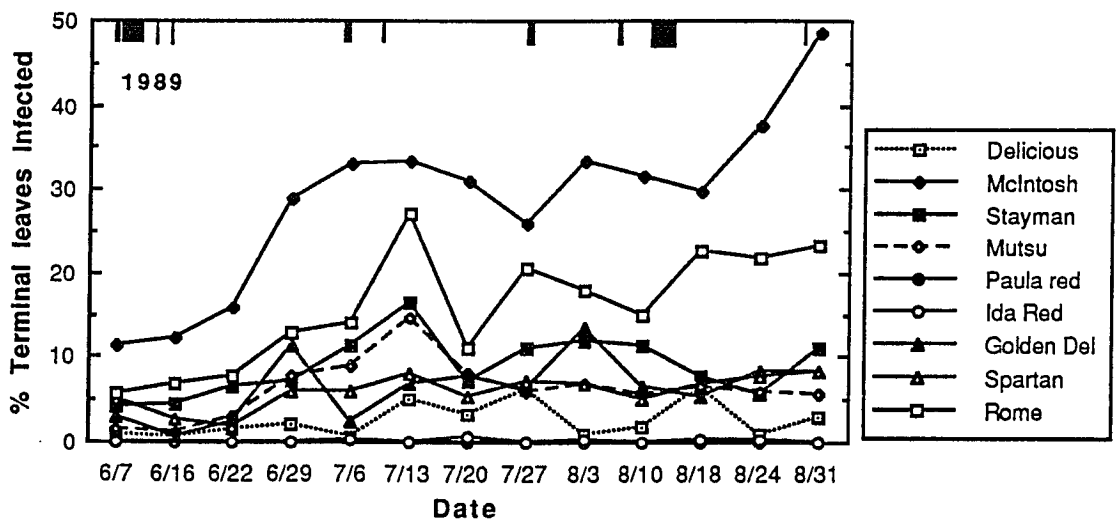


Fig. 10. Incidence of apple scab caused by *Venturia inaequalis* on extension shoot leaves during 1989. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch mark indicates the duration of the infection period.

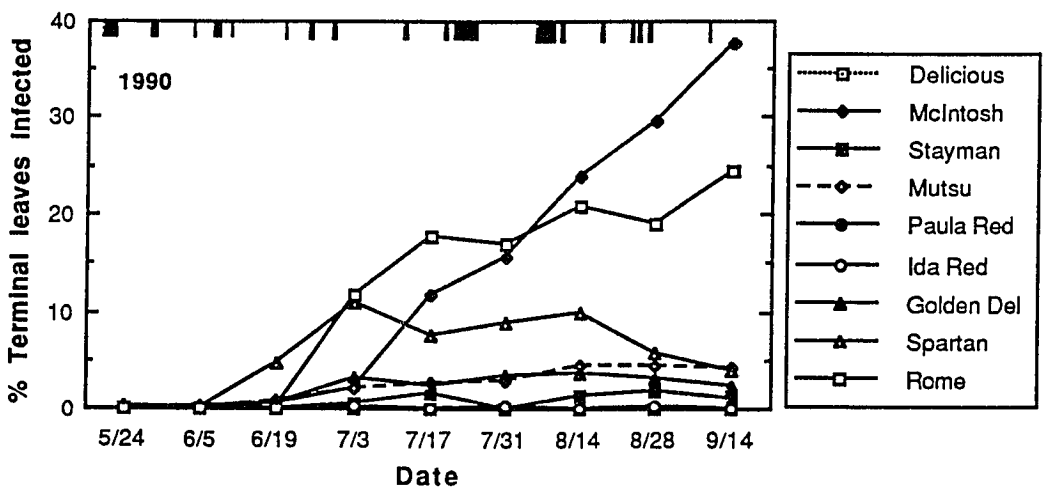


Fig. 11. Incidence of apple scab caused by *Venturia inaequalis* on extension shoot leaves during 1990. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch mark indicates the duration of the infection period.

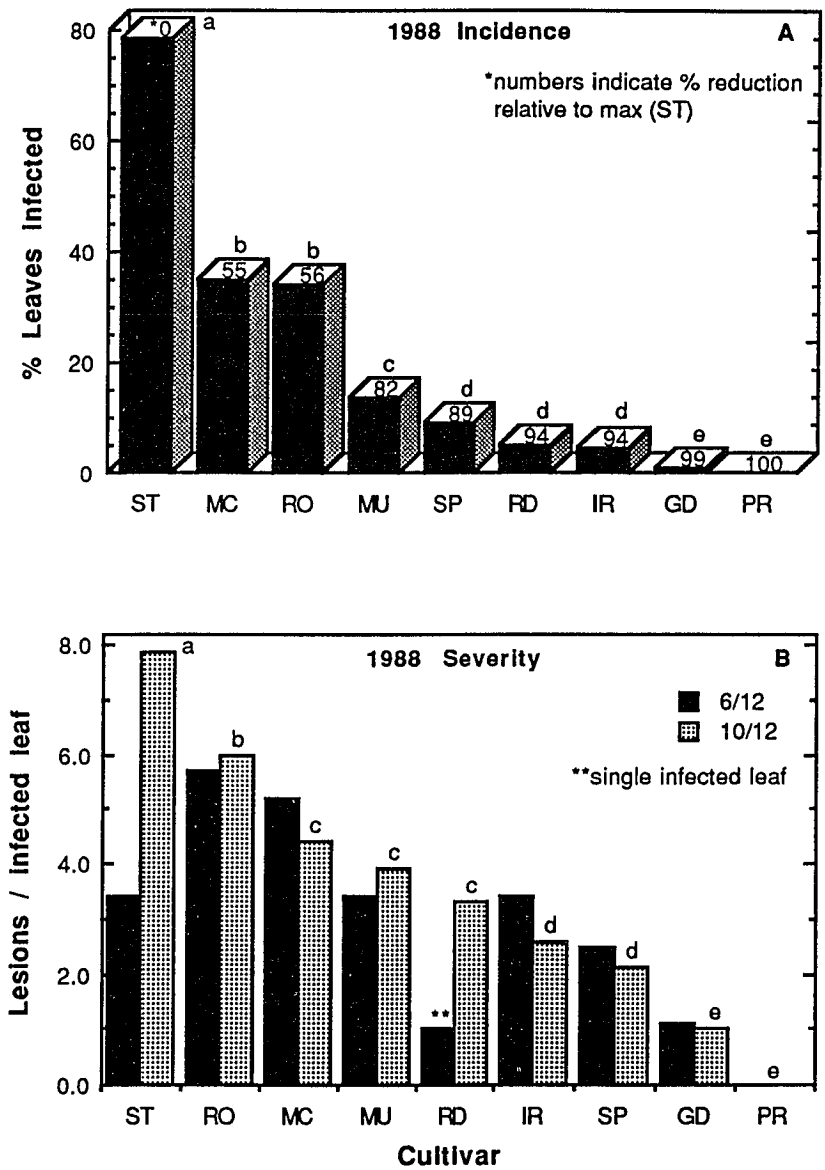


Fig. 12 Apple scab incidence just prior to leaf-fall (**A**) and severity (**B**) at the end of the primary scab season (6/12) and just prior to leaf-fall (10/12) on extension shoot leaves infected with *Venturia inaequalis* in 1988. ST = 'Stayman,' MC = 'McIntosh,' RO = 'Rome,' MU = 'Mutsu,' SP = 'Spartan,' RD = 'Delicious,' IR = 'Ida Red,' GD = 'Golden Delicious,' PR = 'Paula Red.' Columns labeled by the same letter do not differ significantly (P = 0.05).

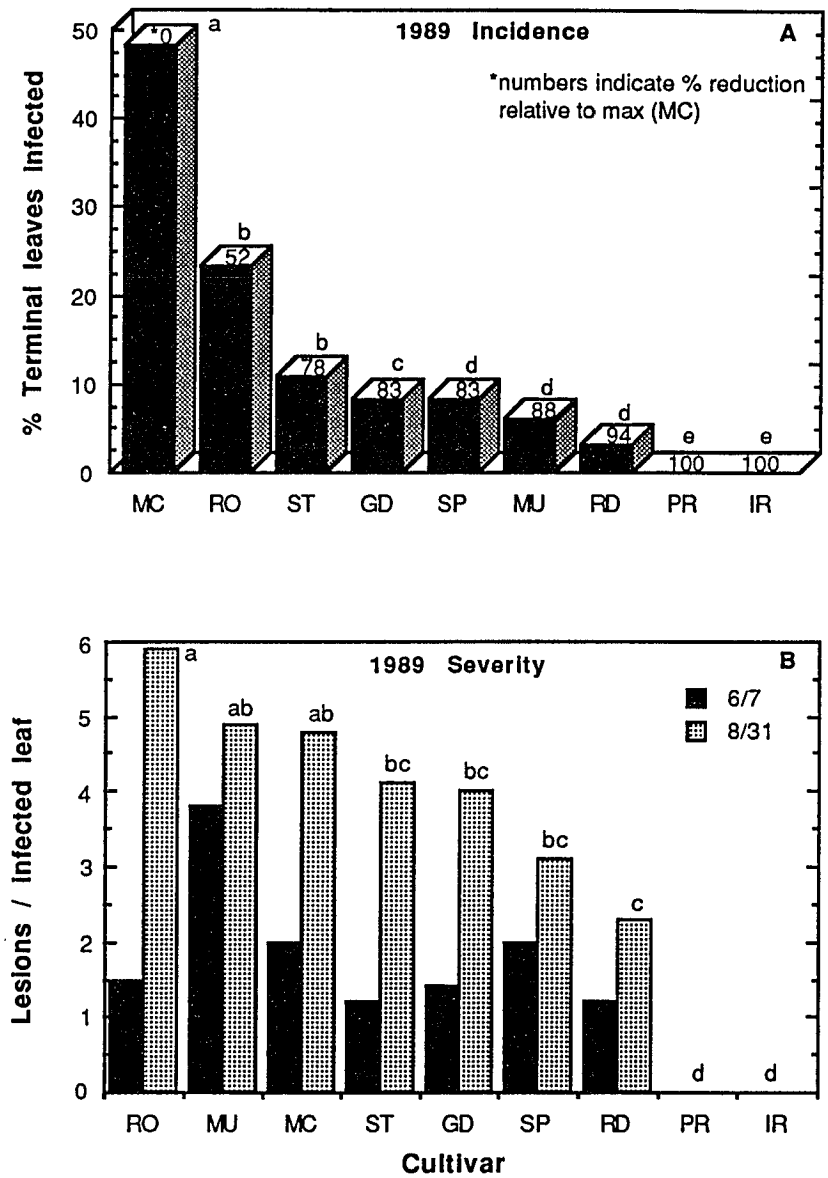


Fig. 13. Apple scab incidence just prior to leaf-fall (**A**) and severity (**B**) at the end of the primary scab season (6/7) and just prior to leaf-fall (8/31) on extension shoot leaves infected with *Venturia inaequalis* in 1989. Columns labeled by the same letter do not differ significantly (P = 0.05). MC = 'McIntosh,' RO = 'Rome,'ST = 'Stayman,' GD = 'Golden Delicious,' SP = 'Spartan,' MU = 'Mutsu,' RD = 'Delicious,' PR = 'Paula Red,' and IR = 'Ida Red.'

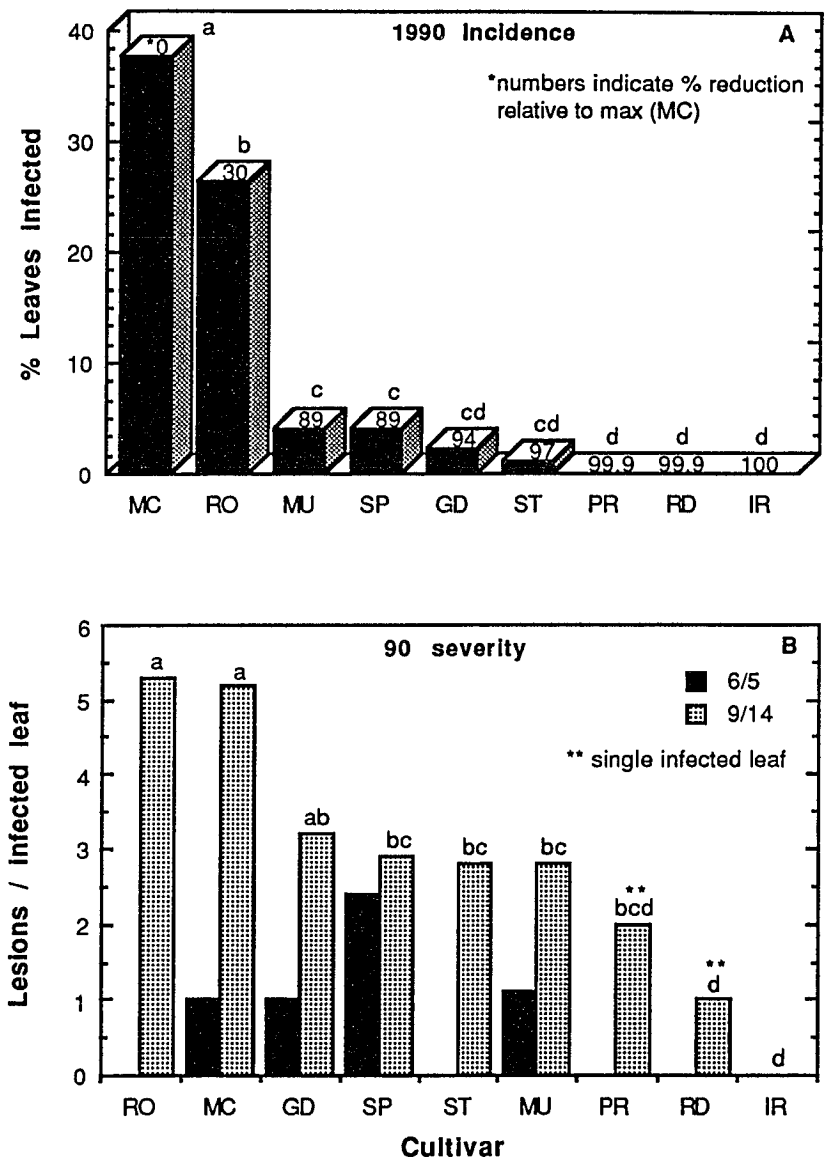


Fig. 14. Apple scab incidence just prior to leaf-fall (**A**) and severity (**B**) at the end of the primary scab season (6/12) and just prior to leaf-fall (9/14) on extension shoot leaves infected with *Venturia inaequalis* in 1990. Columns labeled by the same letter do not differ significantly (P = 0.05). MC = 'McIntosh,' RO = 'Rome,' MU = 'Mutsu,' SP = 'Spartan,' GD = 'Golden Delicious,' ST = 'Stayman,' PR = 'Paula Red,' RD = 'Delicious,' and IR = 'Ida Red.'

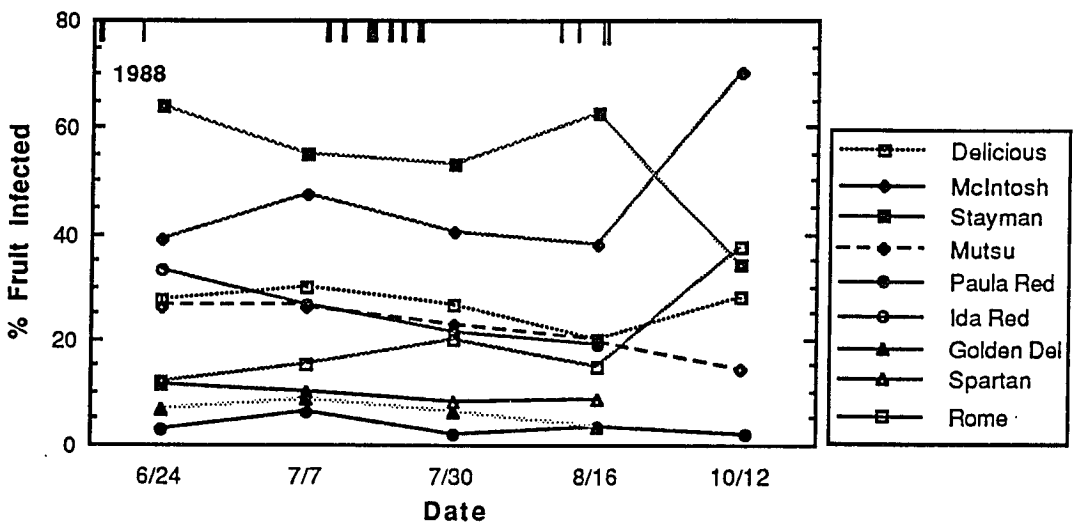


Fig. 15. Incidence of apple scab caused by *Venturia inaequlis* on fruit during 1988. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch marks indicates the duration of the infection period.

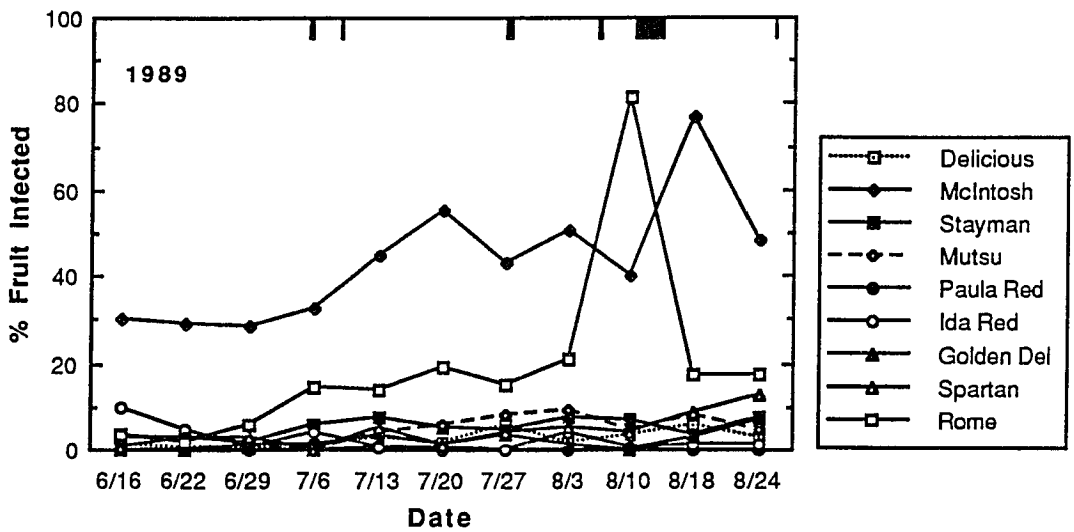


Fig. 16. Incidence of apple scab caused by *Venturia inaequalis* on fruit during 1989. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch marks indicates the duration of the infection period.

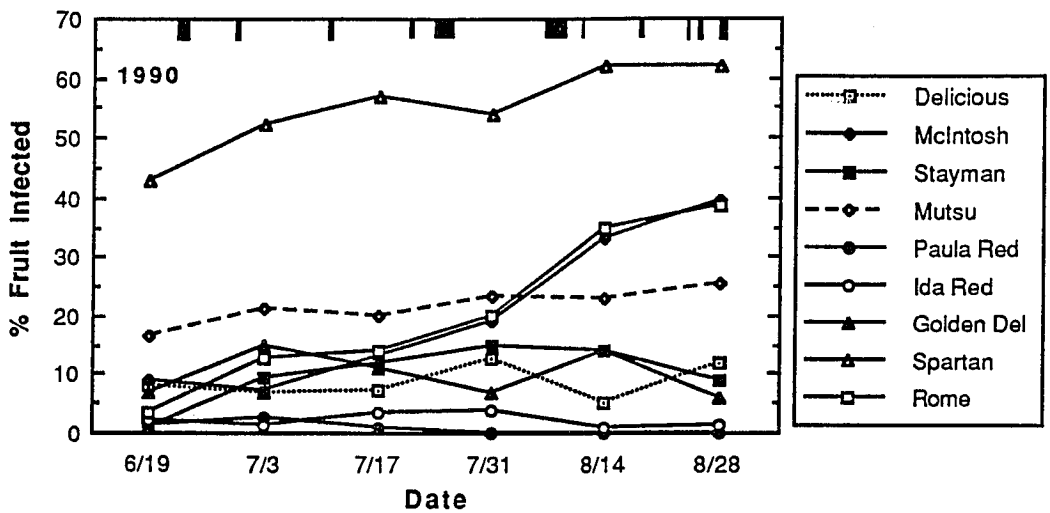


Fig. 17. Incidence of apple scab caused by *Venturia inaequalis* on fruit during 1990. Hatch marks at the top of the graph represent apple scab infection periods. The thickness of the hatch marks indicates the duration of the infection period.

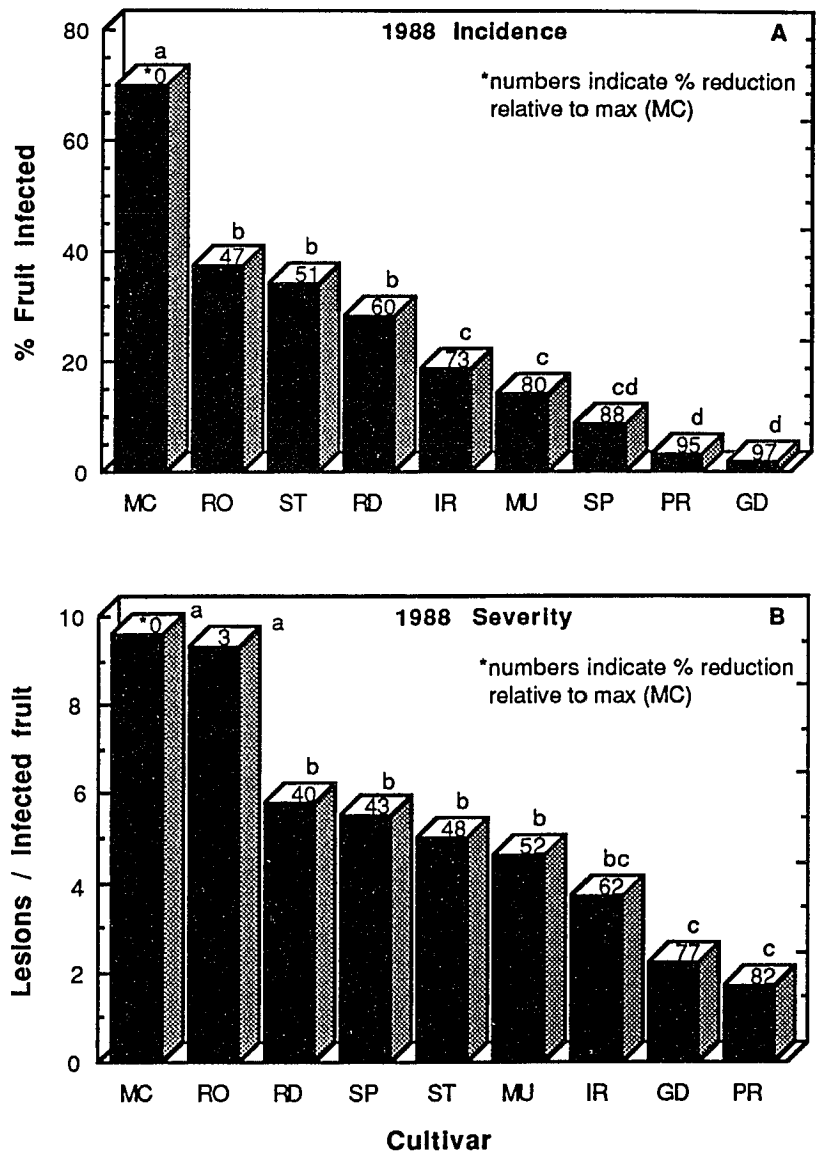


Fig. 18 Apple scab incidence (A) and severity (B) caused by *Venturia inaequalis*, on fruit assessed on 12 Oct, 1988. MC = 'McIntosh,' RO = 'Rome,' ST = 'Stayman,' RD = 'Delicious,' IR = 'Ida Red,' MU = 'Mutsu,' SP = 'Spartan,' PR = 'Paula Red,' and GD = 'Golden Delicious.' Columns labeled by the same letter do not differ significantly (P = 0.05).

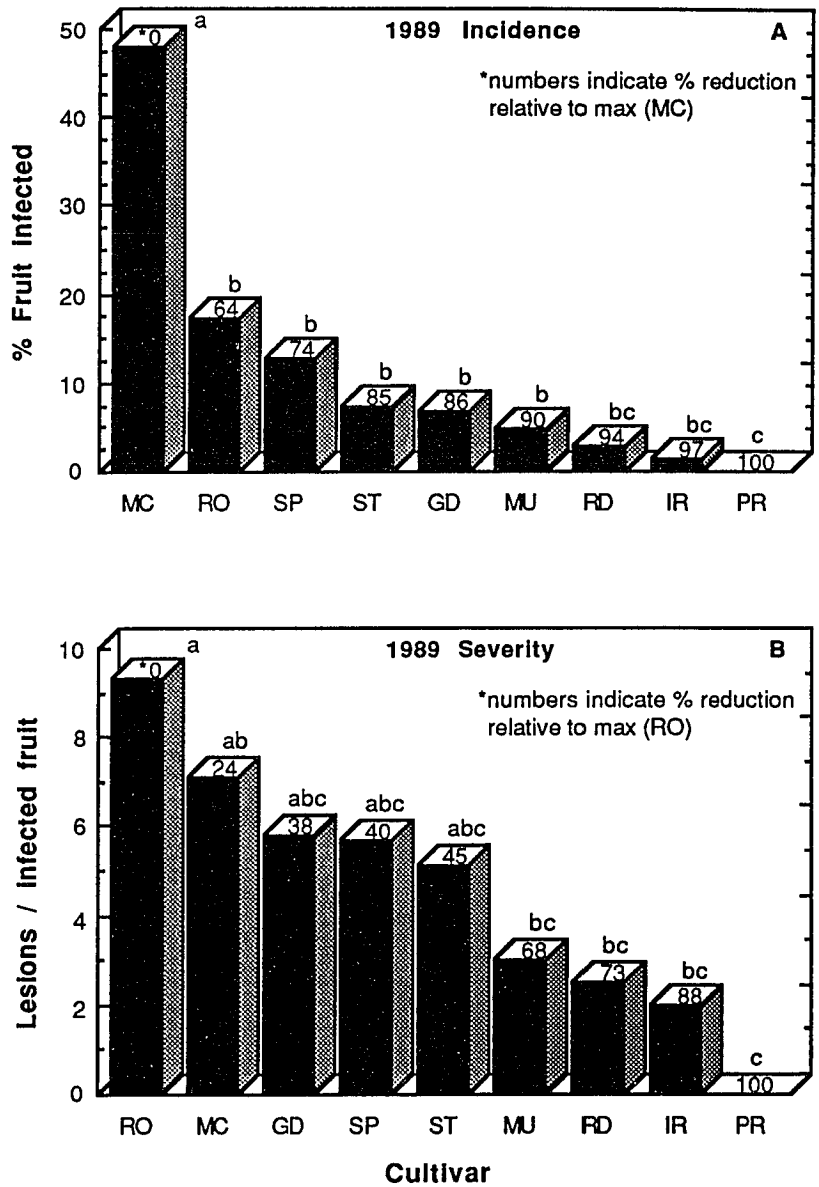


Fig. 19 Apple scab incidence (A) and severity (B) caused by *Venturia inaequalis*, on fruit assessed on 24 Aug, 1989. MC = 'McIntosh,' RO = 'Rome,' SP = 'Spartan,'ST = 'Stayman,' GD = 'Golden Delicious.' MU = 'Mutsu,' RD = 'Delicious,' IR = 'Ida Red,' and PR = 'Paula Red. Columns labeled by the same letter do not differ significantly (P = 0.05).

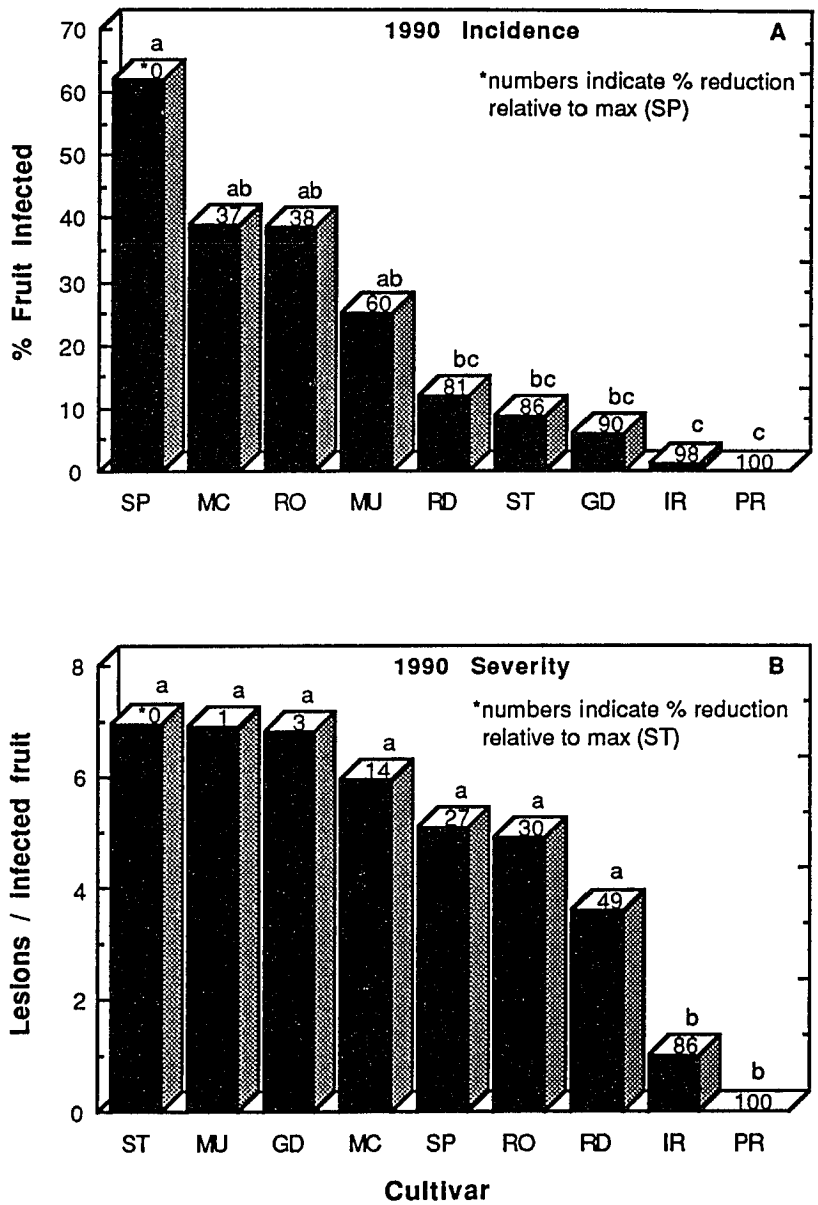
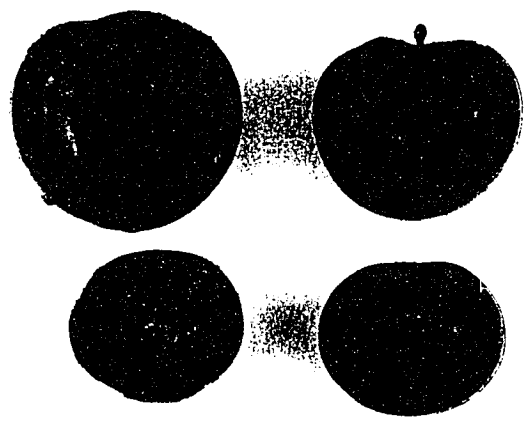


Fig. 20 Apple scab incidence (**A**) and severity (**B**) caused by *Venturia inaequalis*, on fruit assessed on 28 Aug, 1990. SP = 'Spartan,'MC = 'McIntosh,' RO = 'Rome,' MU = 'Mutsu,' RD = 'Delicious,' ST = 'Stayman,' GD = 'Golden Delicious,' IR = 'Ida Red,' and PR = 'Paula Red. Columns labeled by the same letter do not differ significantly (P = 0.05).



Spartan

Fig. 21. Scabbed fruit from the cultivar 'Spartan' infected with *Venturia inaequalis*.

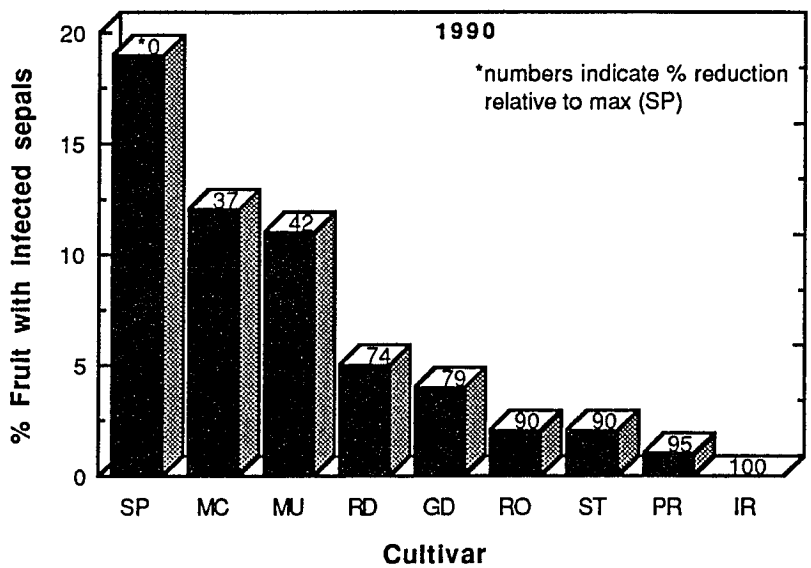


Fig. 22. Incidence of sepal infections caused by *Venturia inaequalis*, on 100 fruit examined for each cultivar at the fruit-set stage of fruit development in 1990. SP = 'Spartan,' MC = 'McIntosh,' MU = 'Mutsu,' RD = 'Delicious,' GD = 'Golden Delicious,' RO = 'Rome,' ST = 'Stayman,' PR = 'Paula Red,' and IR = 'Ida Red.'

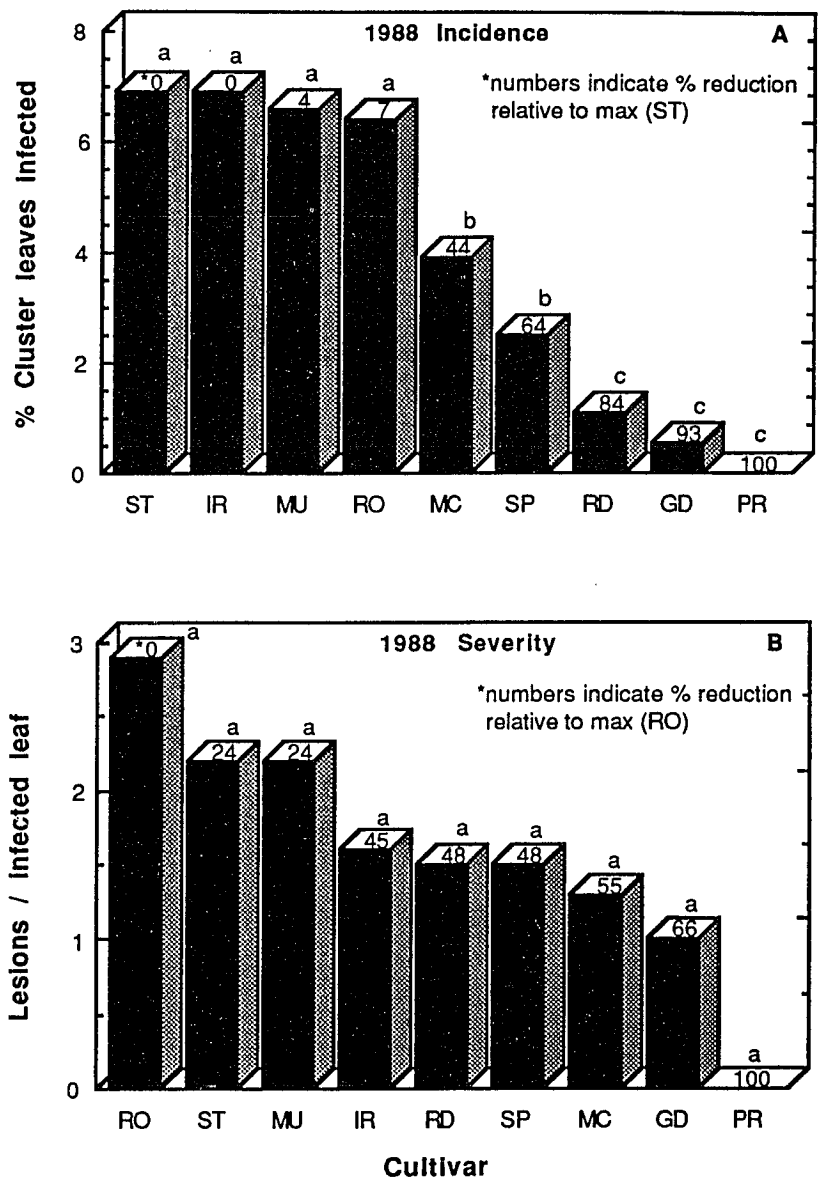


Fig. 23. Incidence (A) and severity (B) of apple scab infections caused by *Venturia inaequalis*, on spur leaves on 10 Jun, 1988. ST = 'Stayman,' IR = 'Ida Red,' MU = 'Mutsu,' RO = 'Rome,' MC = 'McIntosh,' SP = 'Spartan,' RD = 'Delicious,' GD = 'Golden Delicious,' PR = 'Paula Red.' Columns labeled by the same letter do not differ significantly (P = 0.05).

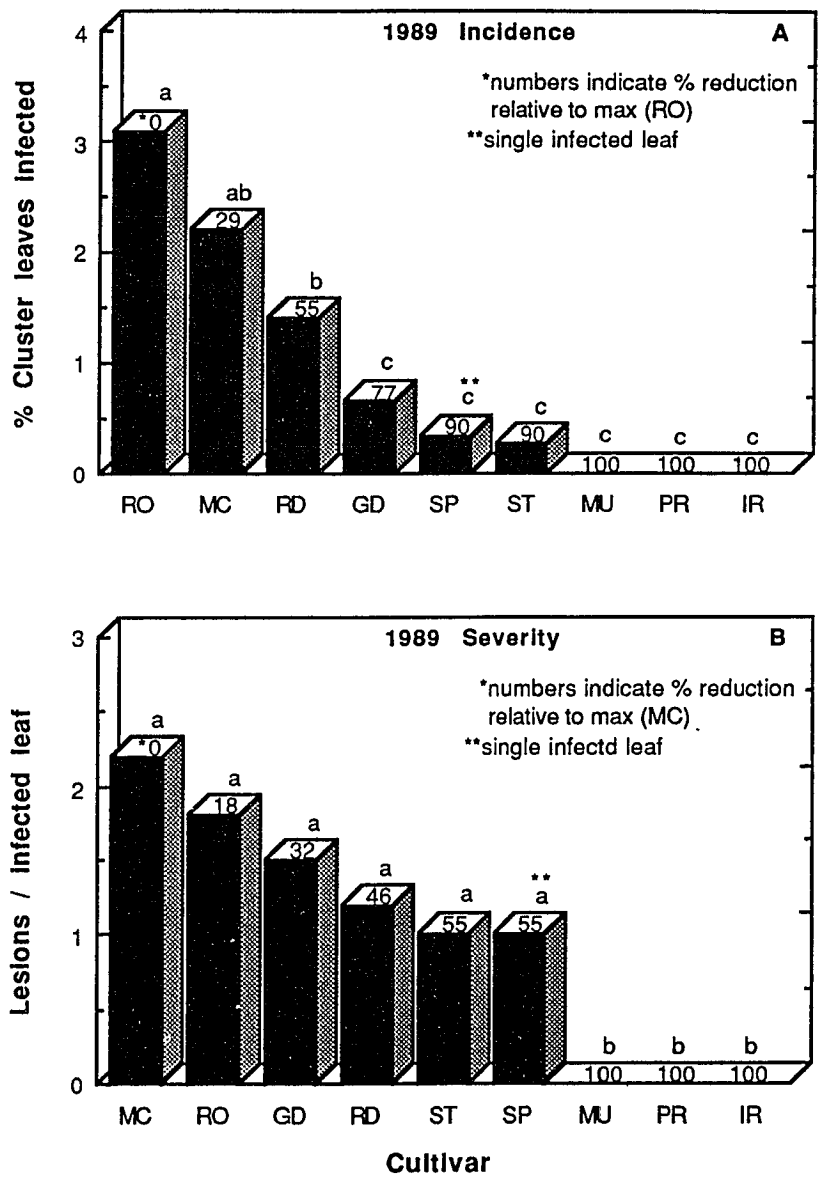


Fig. 24. Incidence (A) and severity (B) of apple scab infections caused by *Venturia inaequalis*, on spur leaves on 16 Jun, 1989. RO = 'Rome,' MC = 'McIntosh,' RD = 'Delicious,' GD = 'Golden Delicious,' SP = 'Spartan,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red.' Columns labeled by the same letter do not differ significantly (P = 0.05)

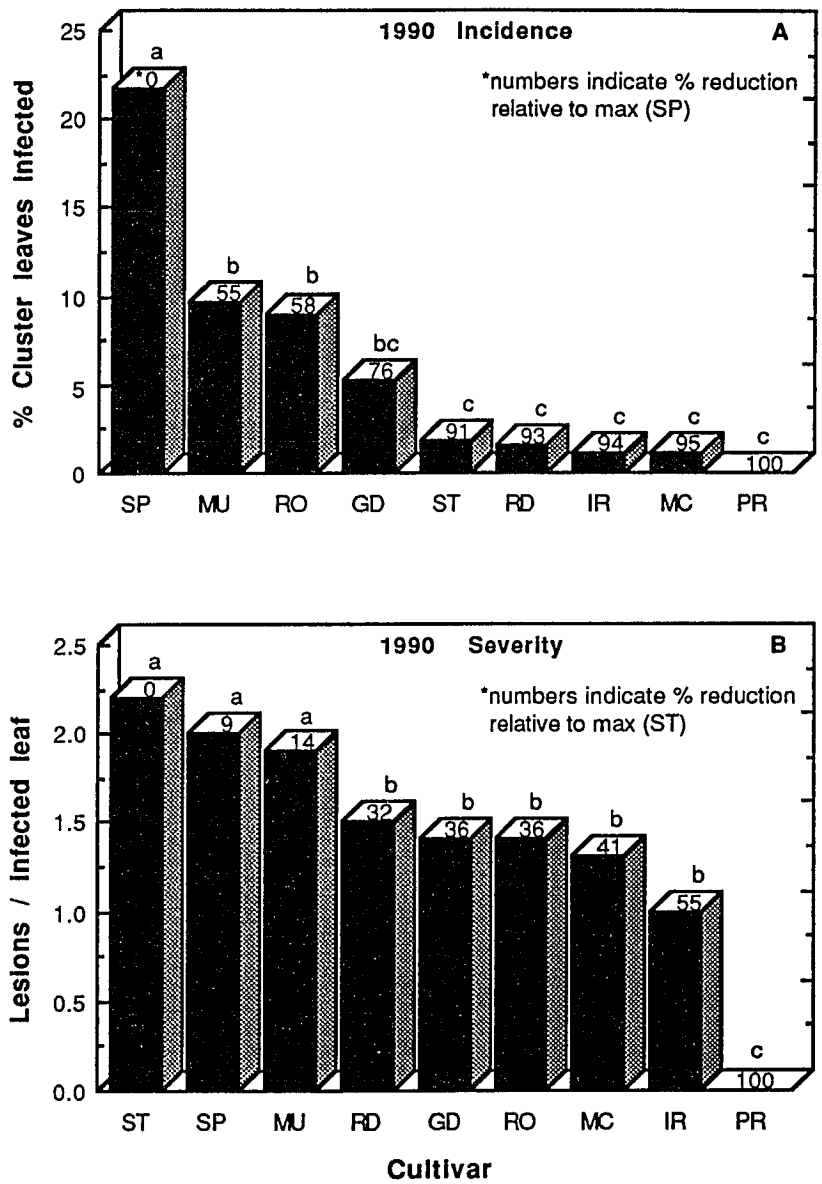


Fig. 25. Incidence (A) and severity (B) of apple scab infections caused by *Venturia inaequalis*, on spur leaves on 19 Jun, 1990. SP = 'Spartan,' MU = 'Mutsu,' RO ='Rome,' GD = 'Golden Delicious,' ST ='Stayman,' RD = 'Delicious,' IR = 'Ida Red,' MC = 'McIntosh,' PR = 'Paula Red.' Columns labeled by the same letter do not differ significantly (P = 0.05).

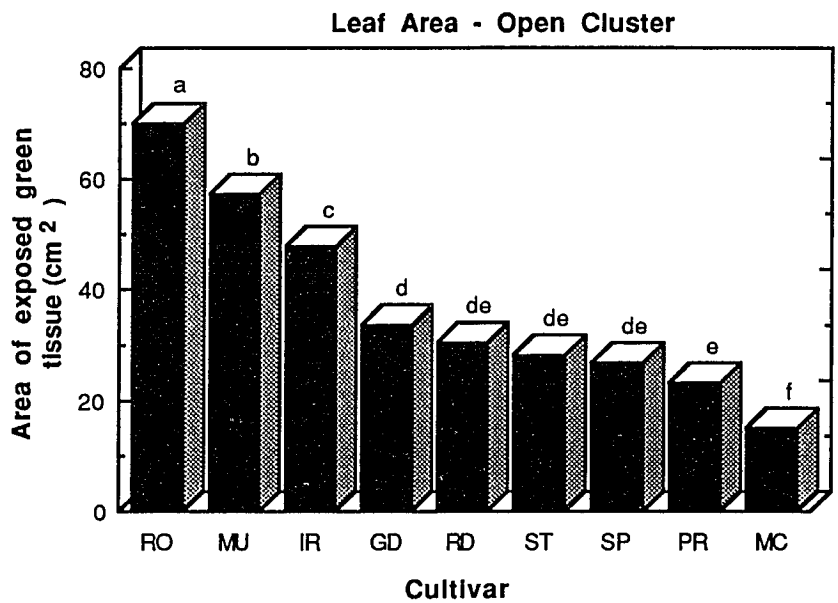


Fig. 26. Area (cm^2) of exposed green tissue for all leaves at the open-cluster stage of fruit bud development. Means labeled by the same letter do not differ significantly (P = 0.05). RO = 'Rome,' MU = 'Mutsu,' IR = 'Ida Red,' GD = 'Golden Delicious,' RD = 'Delicious,' ST = 'Stayman,' SP = 'Spartan,' PR = 'Paula Red,' and MC = 'McIntosh.'

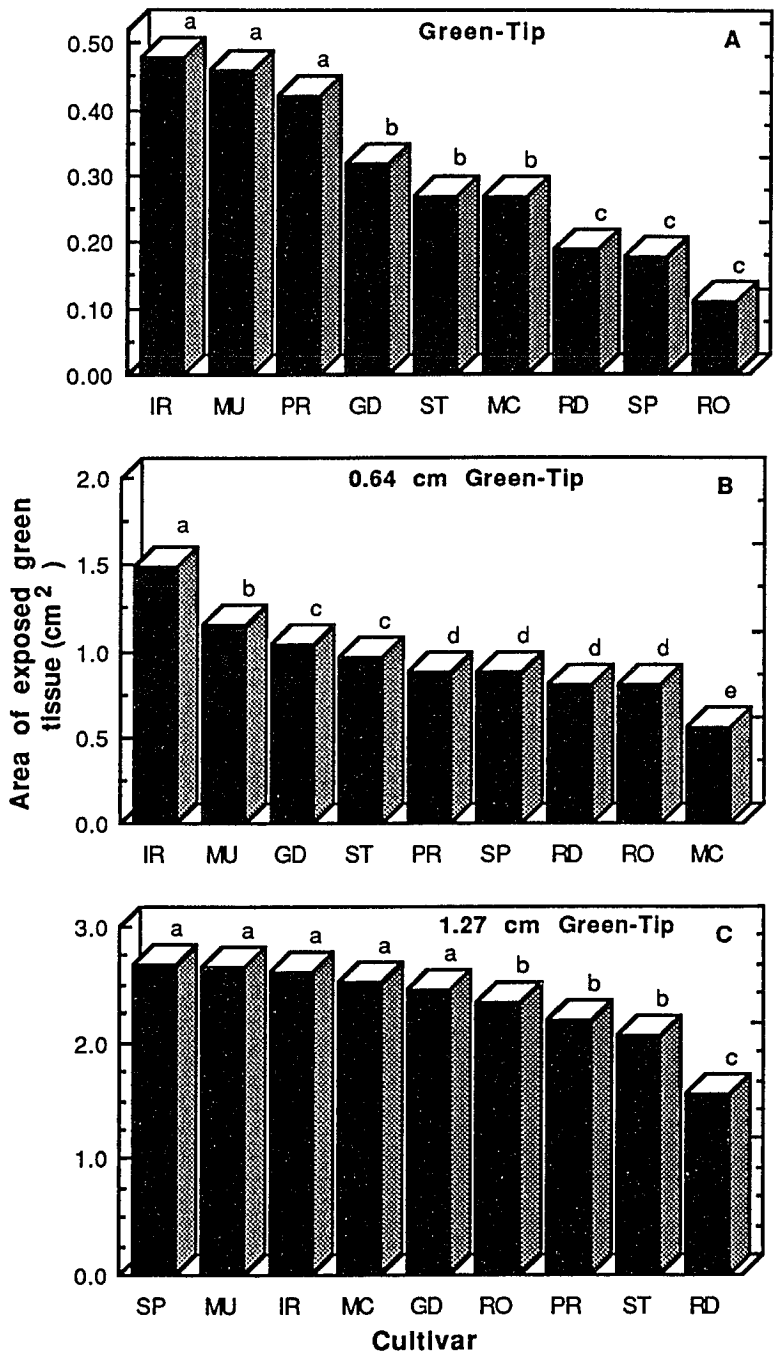


Fig. 27. Area (cm^2) of green tissue exposed at different stages of fruit bud development. (A) green-tip, (B) 0.64 cm (1/4") green-tip, and (C) 1.27 cm (1/2"). Means labeled by the same letter do not differ significantly (P = 0.05). IR = 'Ida Red,' MU = 'Mutsu,' PR = 'Paula Red', GD = 'Golden Delicious,' ST = 'Stayman,' MC = "McIntosh,' RD = 'Delicious,' SP = 'Spartan,' and RO = 'Rome.'

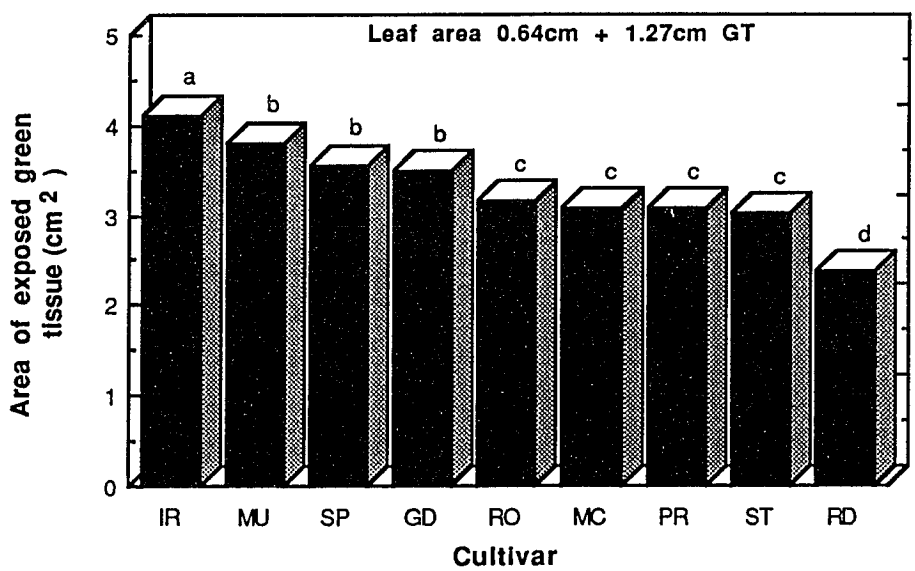


Fig. 28. Area (cm^2) of green tissue exposed when the 0.64 cm green-tip and 1.27 cm green-tip stages of bud development were combined. Means labeled by the same letter do not differ significantly (P = 0.05). IR = 'Ida Red,' MU = 'Mutsu,' SP = 'Spartan,' GD = 'Golden Delicious,' RO = 'Rome,' MC = 'McIntosh,' PR = 'Paula Red,' ST = 'Stayman,' and RD = 'Delicious.'

Table 2. Area of susceptible leaf tissue for the five youngest extension shoot leaves of each cultivar.

	Leaf areas					
Cultivar	Leaf #1[x]	Leaf #2	Leaf #3	Leaf #4	Leaf #5	Leaves 1-5
Mutsu	4.9 b[y, z]	12.8 a	22.6 a	33.4 a	42.7 a	116.3 a
Rome	4.1 b	12.0 a	19.5 b	24.7 b	27.6 b	88.0 b
Spartan	5.8 a	13.9 a	18.5 b	23.4 b	27.1 b	85.7 b
Golden Del	3.6 c	8.0 b	14.3 c	21.4 b	27.4 b	74.7 c
Paula Red	3.8 c	9.0 b	13.9 c	20.1 b	23.7 c	70.4 c
Stayman	2.4 d	6.2 c	12.9 c	19.7 c	27.3 b	68.5 c
McIntosh	1.9 e	4.9 d	9.3 d	15.1 c	19.0 c	50.1 d
Ida Red	2.2 d	3.6 d	6.1 d	9.3 d	13.3 d	34.4 e
Delicious	2.0 e	3.6 d	6.0 d	9.3 d	12.6 d	33.5 e

[x] Leaves numbered by position, with #1 corresponding to the youngest leaf on the extension shoot terminal.

[y] Mean leaf area (cm^2) determined from 30 leaf samples.

[z] Means within the same column followed by the same letter are not significantly different (P = 0.05).

Table 3. Relationship between leaf area[a] and leaf width, leaf length, the product of leaf width and leaf length, leaves/shoot[b], and shoot length[b] determined by linear regression analysis.

Cultivar	Coefficient of determination (R^2)				
	Leaf width	Leaf length	Leaf W x L	Leaves/shoot	Shoot length
Delicious	0.85	0.87	0.98	0.06	0.12
McIntosh	0.93	0.92	0.99	0.69	0.40
Stayman	0.90	0.93	0.99	0.39	0.16
Mutsu	0.91	0.89	0.99	0.74	0.47
Paula Red	0.90	0.87	0.99	0.47	0.49
Ida Red	0.93	0.90	0.99	0.61	0.13
Golden Delicious	0.90	0.92	0.99	0.71	0.39
Spartan	0.93	0.91	0.99	0.19	0.05
Rome	0.90	0.91	0.99	0.47	0.12

[a] Leaf areas determined with a photoelectric leaf area meter.

[b] Compared to total leaf area for all extension shoot leaves.

Fig. 29. The frequency of apple fruit bud phenophases on nine apple cultivars on each sampling date in 1987. The growth stage corresponding to each phenophase is; 1 = dormant, 2 = silver-tip, 3 = green-tip, 4 = 0.64 cm green-tip, 5 = 1.27 cm green-tip, 6 = 1-2 cluster leaves expanded, 7 = 3-5 cluster leaves expanded, 8 = tight cluster, 9 = 1 bud away from tight cluster, 10 = 2-3 buds away from tight cluster, 11 = open cluster, 12 = pink, 13 = bloom, 14 = 1-2 blossoms' petals fallen, 15 = petal-fall, and 16 = fruit-set. RD = 'Red Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'

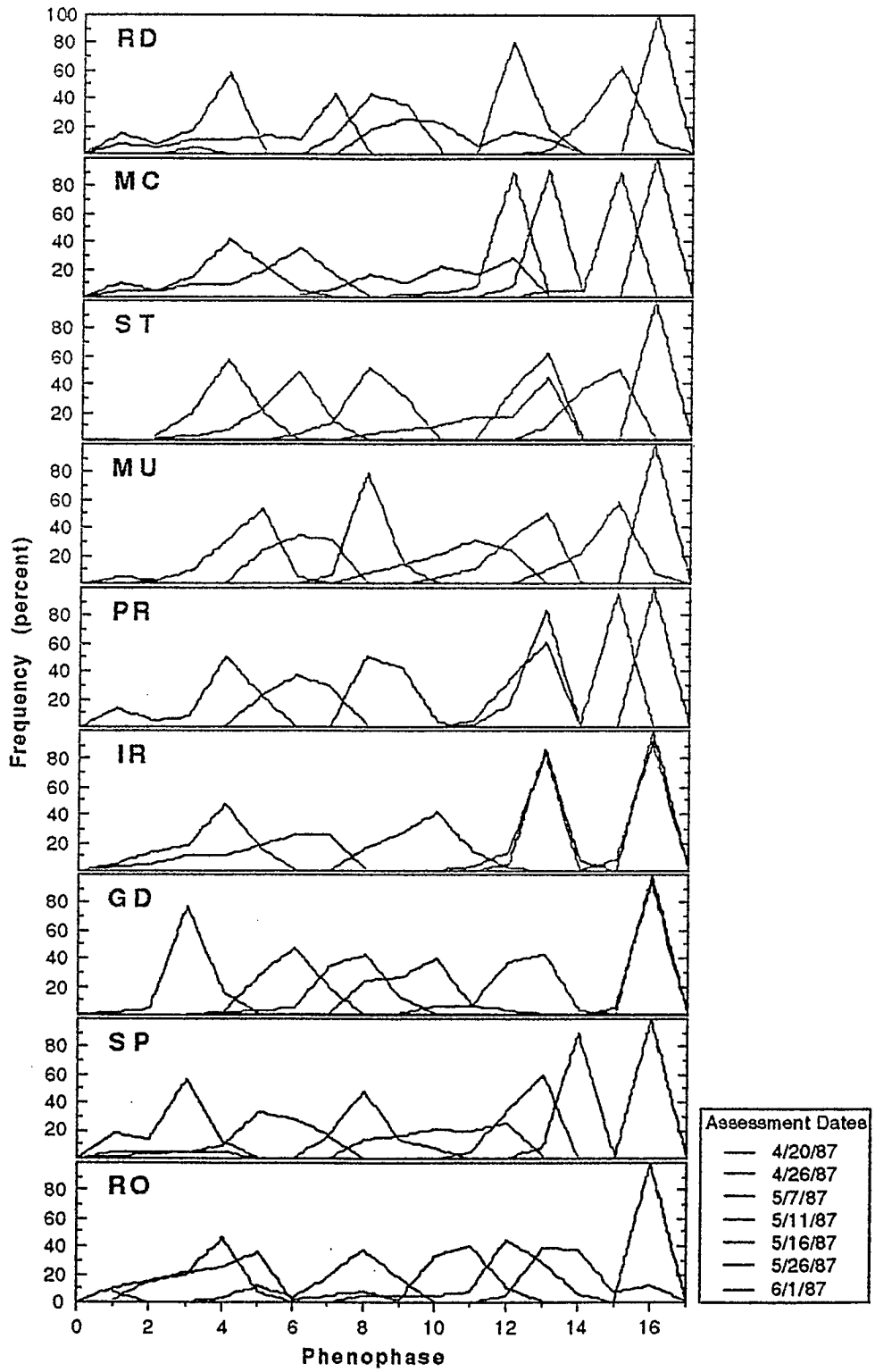


Fig. 30. The frequency of apple fruit bud phenophases on nine apple cultivars on each sampling date in 1989. The growth stage corresponding to each phenophase is; 1 = dormant, 2 = silver-tip, 3 = green-tip, 4 = 0.64 cm green-tip, 5 = 1.27 cm green-tip, 6 = 1-2 cluster leaves expanded, 7 = 3-5 cluster leaves expanded, 8 = tight cluster, 9 = 1 bud away from tight cluster, 10 = 2-3 buds away from tight cluster, 11 = open cluster, 12 = pink, 13 = bloom, 14 = 1-2 blossoms' petals fallen, 15 = petal-fall, and 16 = fruit-set. RD = 'Red Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'

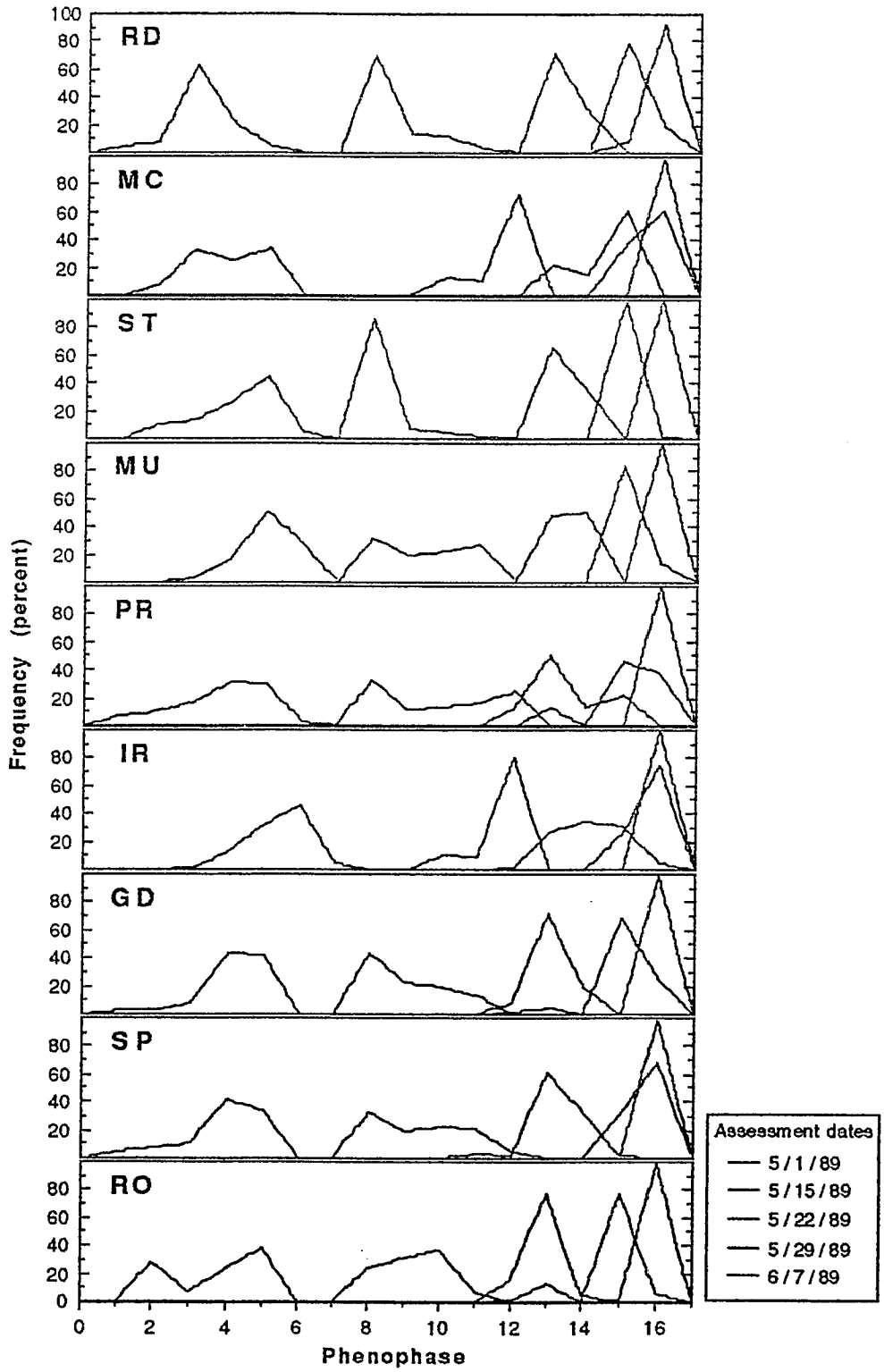


Fig. 31. The frequency of apple fruit bud phenophases on nine apple cultivars on each sampling date in 1990. The growth stage corresponding to each phenophase is; 1 = dormant, 2 = silver-tip, 3 = green-tip, 4 = 0.64 cm green-tip, 5 = 1.27 cm green-tip, 6 = 1-2 cluster leaves expanded, 7 = 3-5 cluster leaves expanded, 8 = tight cluster, 9 = 1 bud away from tight cluster, 10 = 2-3 buds away from tight cluster, 11 = open cluster, 12 = pink, 13 = bloom, 14 = 1-2 blossoms' petals fallen, 15 = petal-fall, and 16 = fruit-set. RD = 'Red Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'

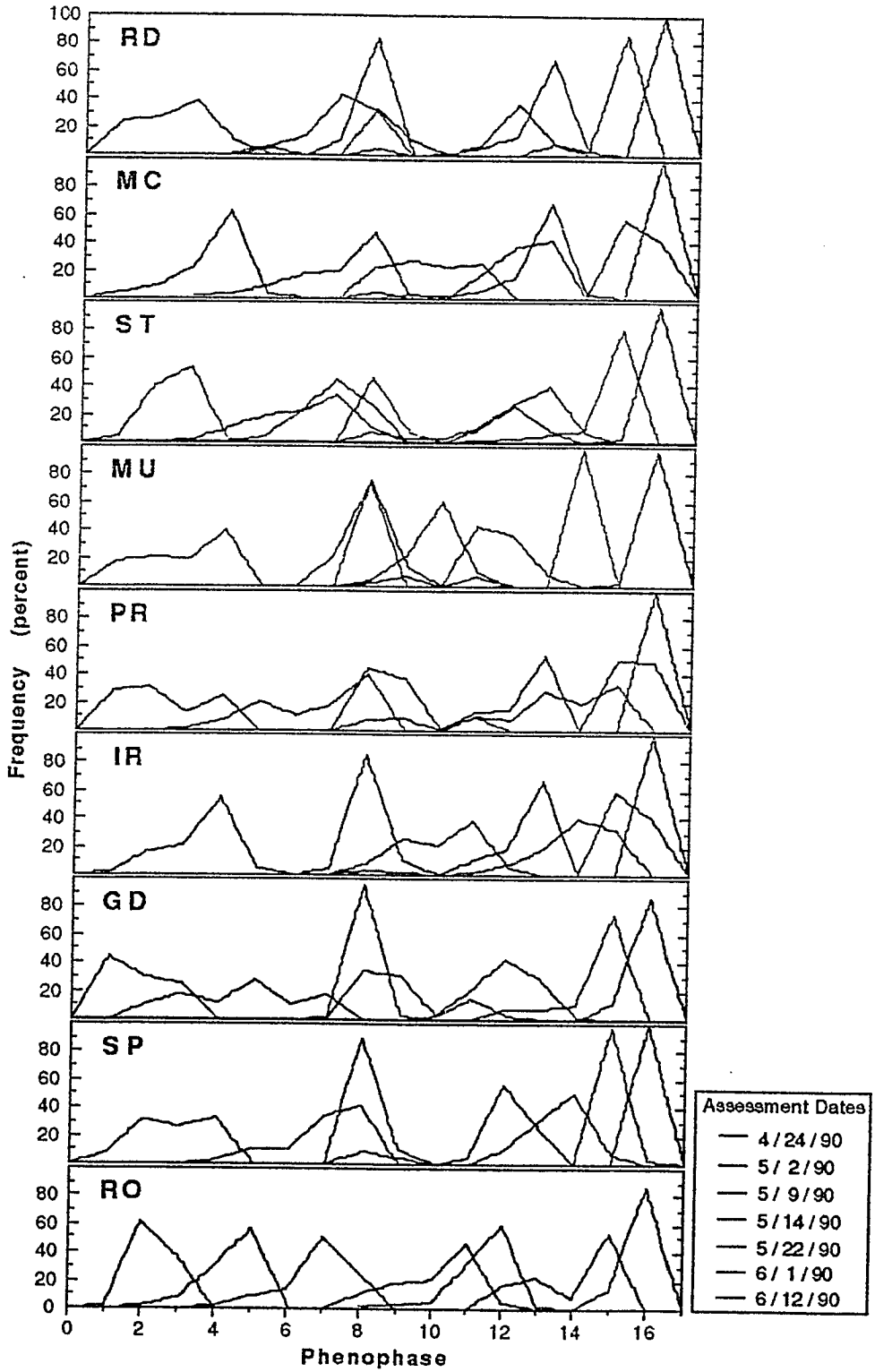


Table 4. Extension shoot growth; leaves/shoot, date of terminal bud set, and rate of shoot growthfor nine apple cultivars in 1991.

Cultivar	Maximum number of leaves/shoot [a, b]	Date of terminal bud set [c]	Rate of shoot growth [d]
Delicious	23	Jul 27	1.7
McIntosh	25	Aug 3	1.9
Stayman	22	Aug 31	1.8
Mutsu	21	Jul 27	1.4
Paula Red	21	Jul 20	1.3
Ida Red	23	Jul 27	1.6
Golden Delicious	21	Aug 24	1.4
Spartan	23	Aug 17	1.4
Rome	23	Aug 17	1.7

[a] Means based on measurements from 20 extension shoots.

[b] Means do not differ significantly (P = 0.05).

[c] Date when 100% of the extension shoots set terminal bud.

[d] Number of leaves unfurled/week determined by regression analysis. Rates do not differ significicantly (P = 0.05).

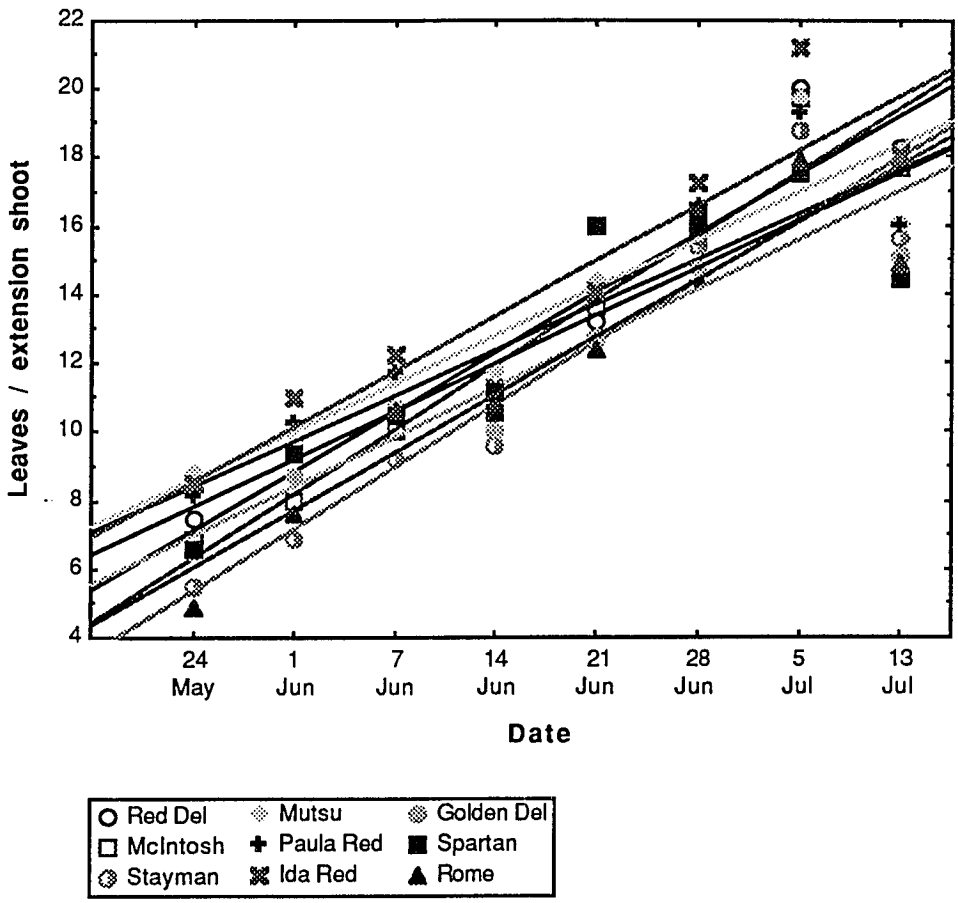


Fig. 32. The growth of extension shoots during the first eight weeks of assessments (just prior to bud-set in 'Paula Red'). Regression lines are fitted to the data for each cultivar. Slope coefficients do not differ significantly (P = 0.05) among the cultivars.

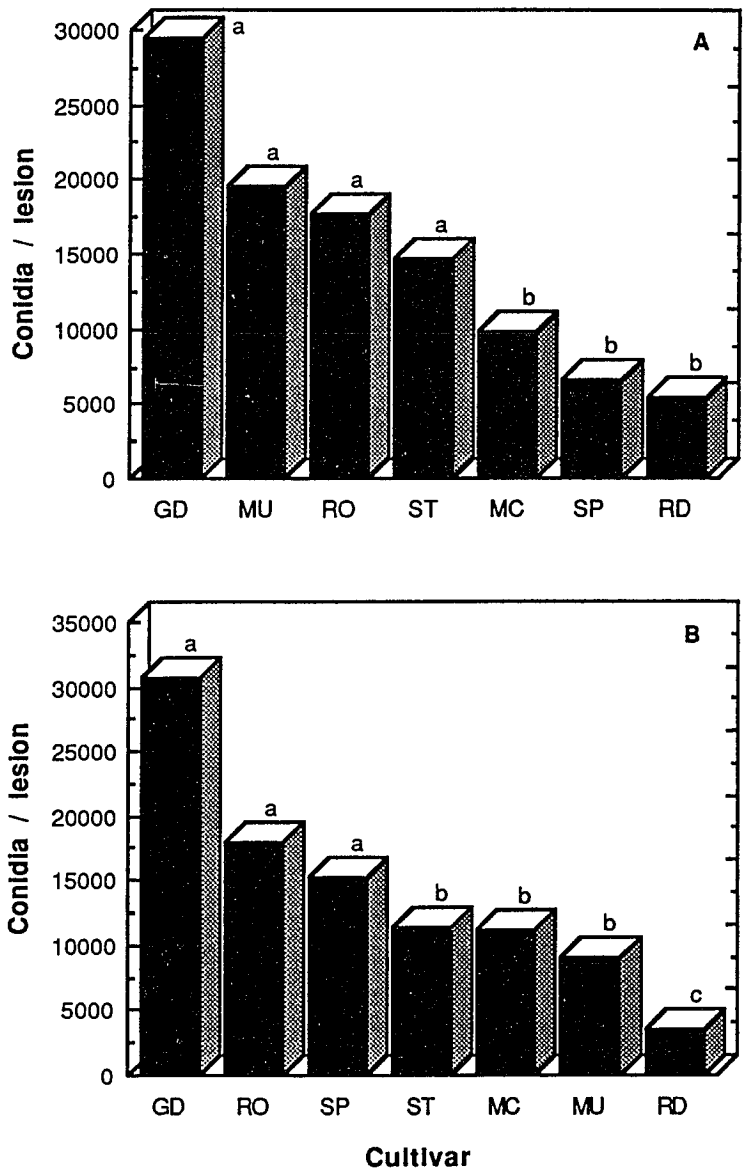


Fig. 33. Total conidia of *Venturia inaequalis* produced per scab lesion on each of seven apple cultivars in 1989 (A) and 1990 (B). Means labeled by the same letter do not differ significantly (P = 0.10). GD = 'Golden Delicious,' MU = 'Mutsu,' RO = 'Rome,' ST = 'Stayman,' MC = 'McIntosh,' SP = 'Spartan,' and RD = 'Delicious.'

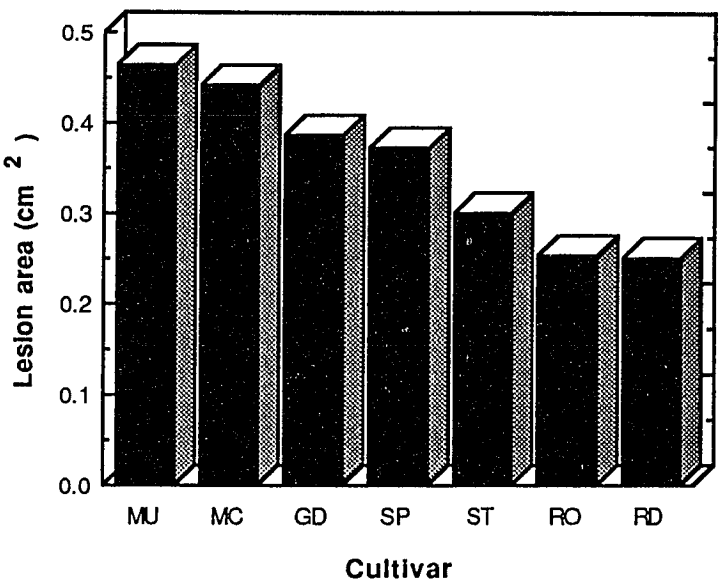


Fig. 34. Relative area (cm^2) of scab lesions caused by *Venturia inaequalis* on each of seven apple cultivars in 1989. Means did not differ significantly (P = 0.10). MU = 'Mutsu,' MC = 'McIntosh,' GD = 'Golden Delicious,' SP = 'Spartan,' ST = 'Stayman,' RO = 'Rome,' and RD = 'Delicious.'

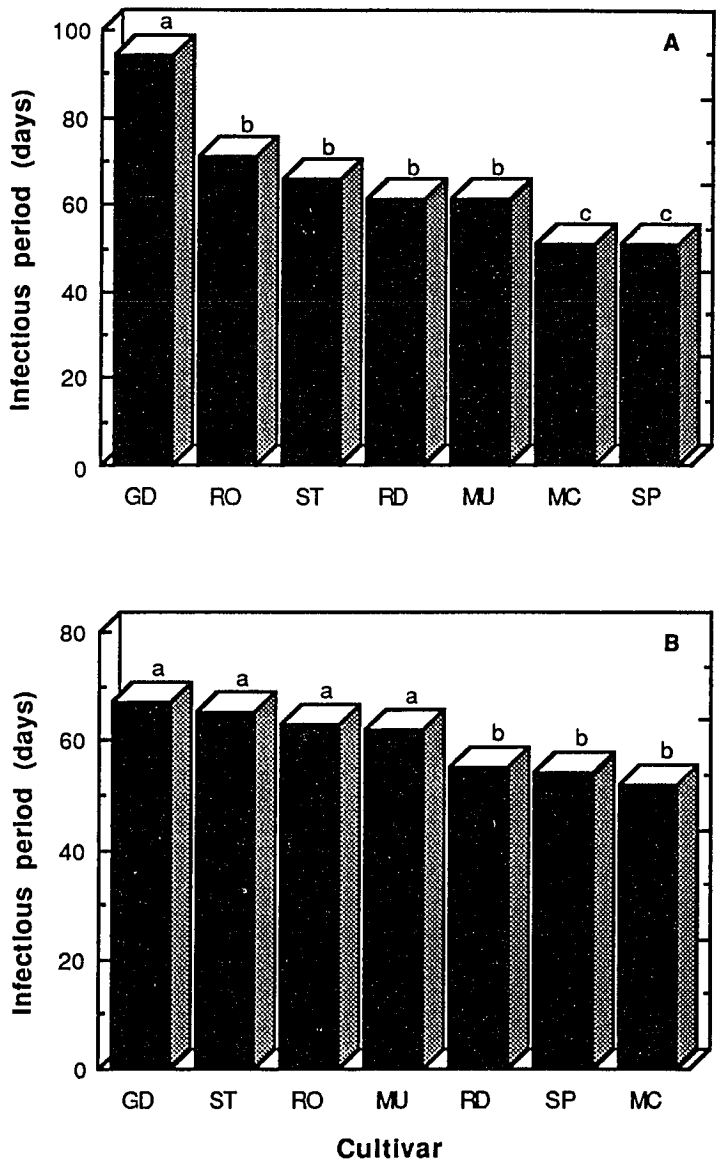


Fig. 35. The number of days between the commencement and cessation of conidium production on scab lesions caused by *Venturia inaequalis* on seven apple cultivars in 1989 (A) and 1990 (B). Means labeled by the same letter do not differ significantly (P = 0.10). GD = 'Golden Delicious,' RO = 'Rome,' ST = 'Stayman,' RD = 'Delicious,' 'MU = 'Mutsu,' MC = 'McIntosh,' and SP = 'Spartan.'

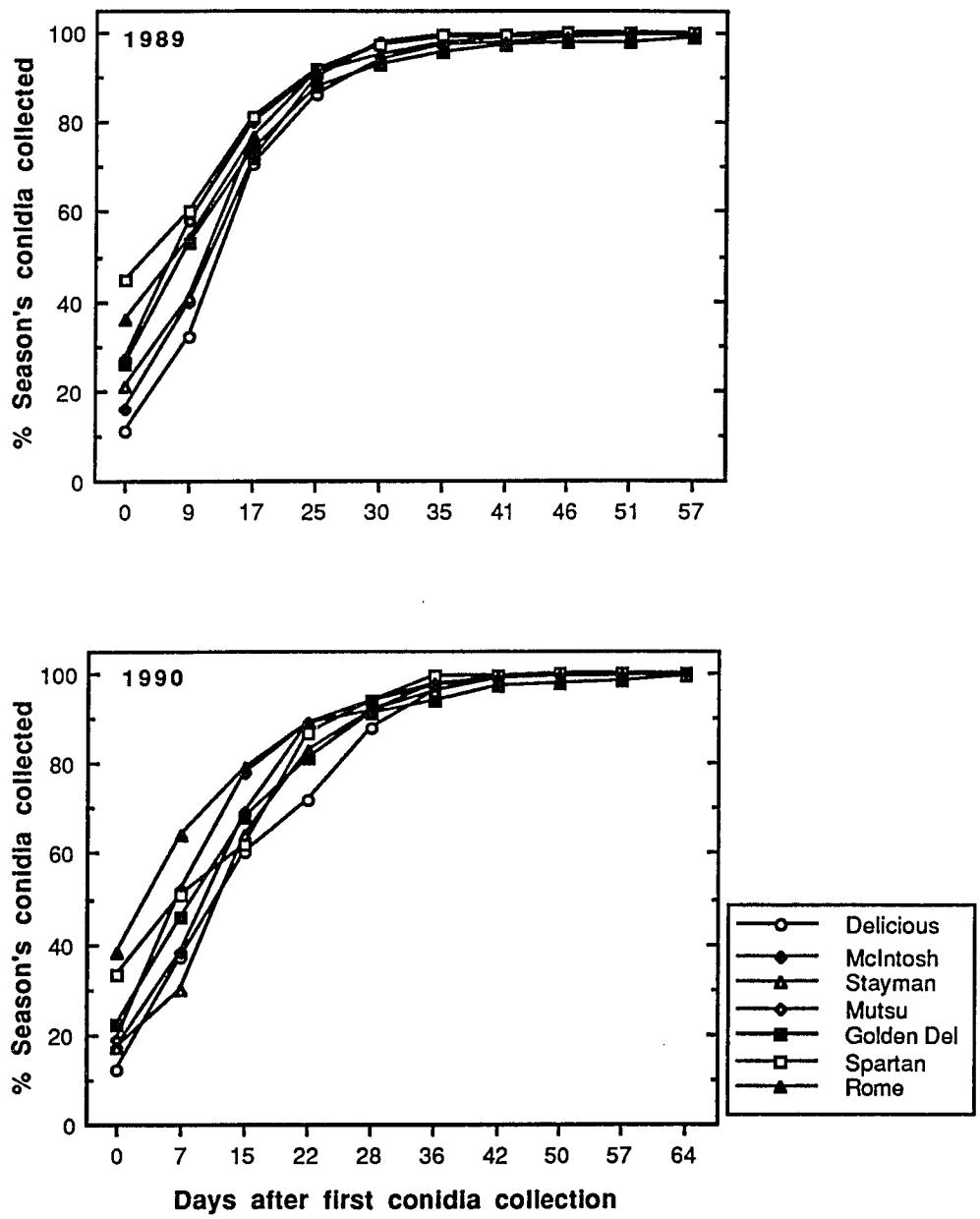


Fig. 36. Conidium production determined from collections made during the secondary apple scab season in 1989 (A) and 1990 (B). The curves represent the cumulative percent season's conida collected for each cultivar.

Table 5. The number of days[z] required to reach 90 and 99 % of the season's total production of conidia of *Venturia inaequalis* on scab lesions on nine apple cultivars in 1989 and in 1990.

Year	Cultivar	90 %	99 %	Days from 90 - 99 %
1989	Stayman	23	33	10
	Mutsu	24	32	8
	Spartan	24	34	10
	McIntosh	26	32	6
	Rome	26	38	12
	Golden Delicious	27	56	29
	Delicious	28	36	8
1990	Rome	23	37	14
	Mutsu	24	37	13
	Stayman	26	39	13
	McIntosh	26	38	12
	Spartan	27	39	12
	Golden Delicious	27	61	34
	Delicious	32	42	10

[z] The number of days from the date that the first conidia collection was made (day 0).

Table 6. The severity, incidence, and incubation period of infections caused by *Venturia inaequalis* on six apple cultivars in 1990 and 1991.

Year	Cultivar	Severity[s]		Incidence[t]		Incubation period[u]	
		Leaf # 1[v]	Leaf # 2[v]	Leaf # 1[v]	Leaf #2[v]	IPS[w]	IPL[x]
1990	Mutsu	12 [y]	4 [y]	80	60	14[y]	18 ab[z]
	Red Del.	12	6	80	50	16	16 a
	Rome	4	4	80	30	18	18 ab
	McIntosh	2	0	30	0	16	16 a
	Paula Red	2	0	20	0	20	23 b
	Golden Del.	2	2	40	30	18	19 ab
1991	Red Del.	42a[z]	6[y]	70	6	12[y]	15[y]
	Rome	37a	21	80	21	10	17
	McIntosh	34a	27	90	27	10	15
	Mutsu	29a	10	70	10	12	14
	Golden Del.	2b	2	30	2	14	16
	Paula Red	---	---	0	0	---	---

[s] Mean number of lesions per infected leaf.

[t] Percent leaves infected of 80 leaves inoculated.

[u] Days from inoculation when lesions first became visible.

[v] Leaf # 1 was the youngest unfurled leaf at the time of inoculation.

[w] Mean latent period when the first lesion became visible on a shoot, regardless of leaf position.

[x] Mean latent period for all infected leaves on 10 shoots.

[y] Means in column do not differ significantly (P = 0.10).

[z] Means followed by the same letter do not differ significantly (P = 0.10).

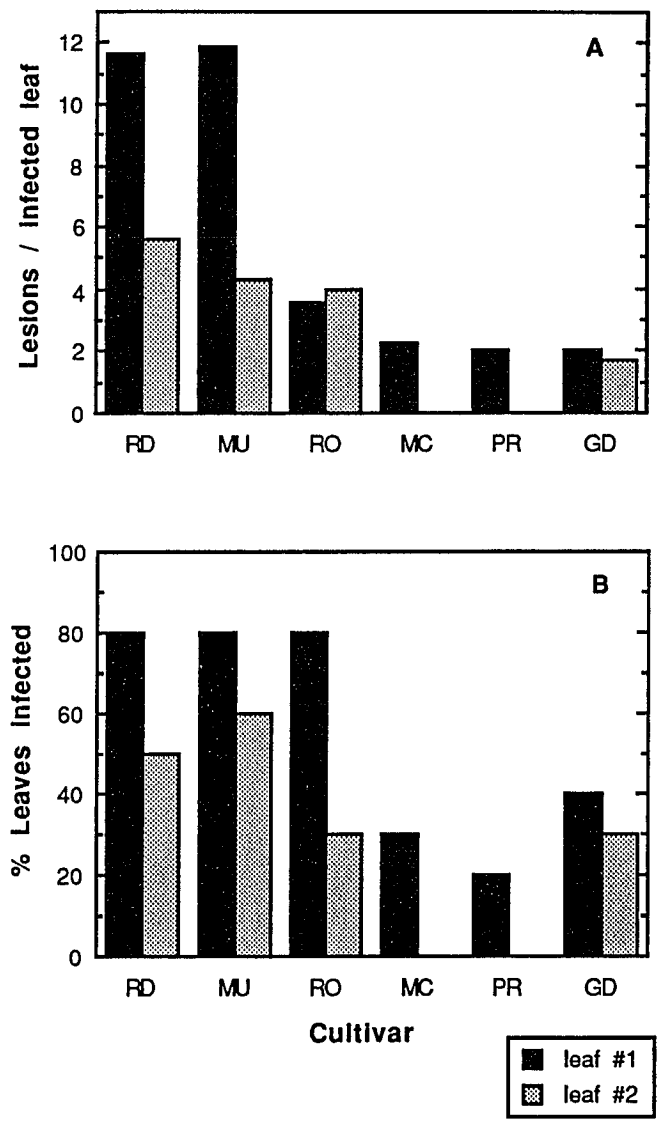


Fig. 37. The severity (A) and incidence (B) of apple scab caused by *Venturia inaequalis* on the youngest two leaves of six apple cultivars in the Smith block in 1990. RD = 'Red Delicious,' MU = 'Mutsu,' RO = 'Rome,' MC = 'McIntosh,' PR = 'Paula Red,' and GD = 'Golden Delicious.'

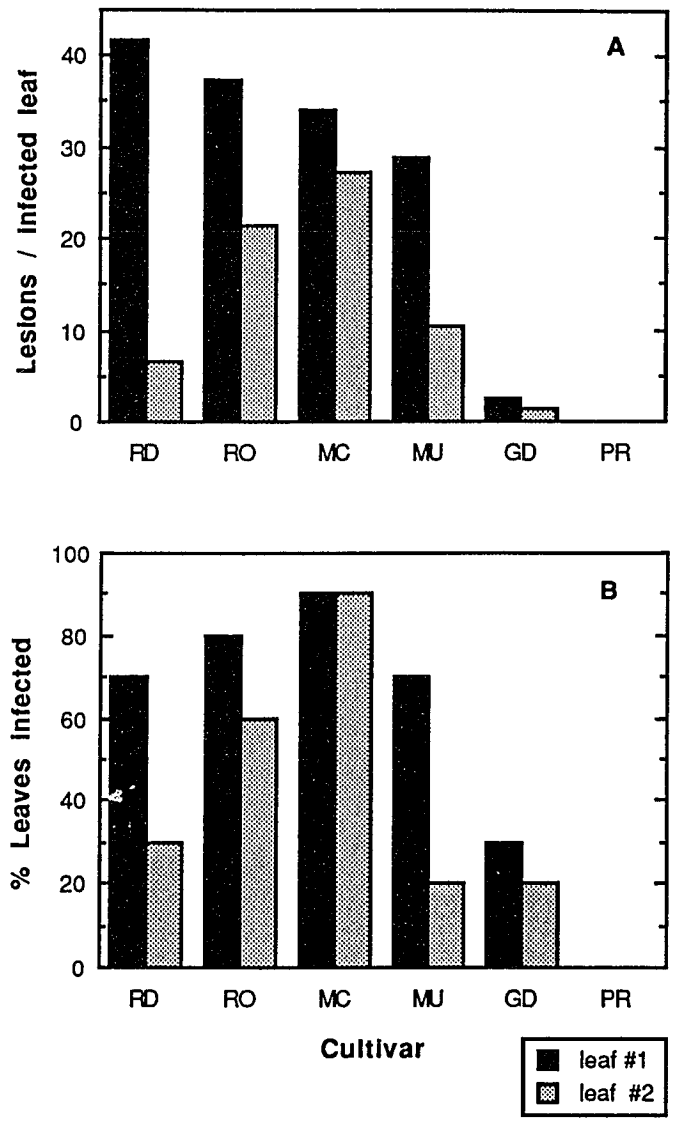


Fig. 38. The severity (A) and incidence (B) of apple scab caused by *Venturia inaequalis* on the youngest two leaves of six apple cultivars in the Smith block in 1991. RD = 'Red Delicious,' RO = 'Rome,' MC = 'McIntosh,' MU = 'Mutsu,' GD = 'Golden Delicious,' and PR = 'Paula Red.'

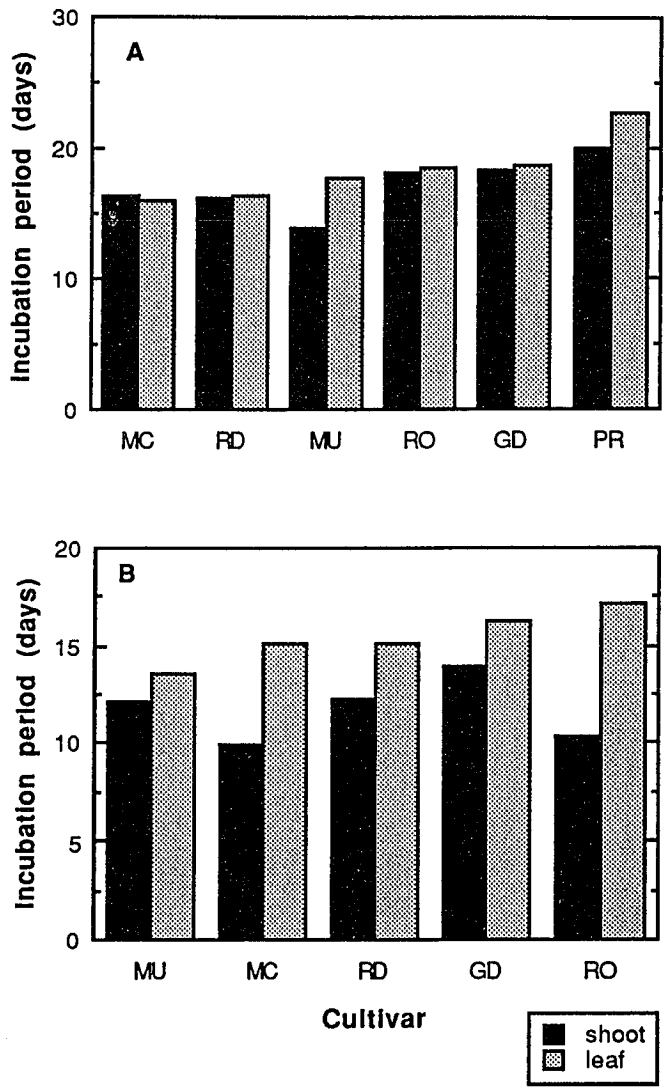


Fig. 39. The number of days between inoculation with *Venturia inaequalis* and the first appearance of sporulating lesions (incubation period) on extension shoots and leaves in the Smith block in 1990 (**A**) and 1991 (**B**) . MU = 'Mutsu,' RD = 'Red Delicious,' MC = 'McIntosh,' RO = 'Rome,' GD = 'Golden Delicious,' and PR = 'Paula Red.'

Table 7. The severity, incidence, and incubation period of infections caused by *Venturia inaequalis* on potted apple trees in 1990 and 1991.

		Severity[s]		Incidence[t]		Incubation period[u]	
Year	Cultivar	Leaf # 1[v]	Leaf # 2[v]	Leaf # 1[v]	Leaf # 2[v]	IPS[w]	IPL[x]
1990	Stayman	16 [y]	10 [y]	86	100	11[y]	11 a[z]
	Mutsu	15	2	22	33	15	16 c
	McIntosh	14	16	50	50	11	12 b
	Spartan	10	10	25	33	11	13 c
	Rome	9	14	80	40	13	14 c
	Red Del.	7	14	25	67	11	12 ab
	Ida Red	7	11	50	50	14	13 b
	Golden Del	5	8	100	37	12	11 ab
	Paula Red	---	---	0	0	---	---
1991	Spartan	8[y]	8[y]	25	33	14 a[z]	15[y]
	McIntosh	8	5	54	55	13 a	15
	Rome	5	12	33	33	11 a	14
	Ida Red	3	1	29	8	16 a	15
	Red Del.	2	2	50	50	19 ab	16
	Stayman	1	4	20	40	16 a	16
	Mutsu	1	4	36	44	15 a	14
	Golden Del.	1	6	22	25	26 b	18
	Paula Red	1*	---	33 *	0	18 ab*	18 *

s Mean number of lesions per infected leaf.
t Percent leaves infected of all leaves inoculated.
u Days from inoculation when lesions first became visible.
v Leaf # 1 was the youngest unfurled leaf at the time of inoculation.
w Mean latent period when the first lesion became visible on a shoot, regardless of leaf position.
x Mean latent period for all infected leaves.
y Means in column do not differ significantly (P = 0.10).
z Means followed by the same letter do not differ significantly (P = 0.10).
* Based on a single infected leaf with one chlorotic lesion that became necrotic six days after first becoming visible.

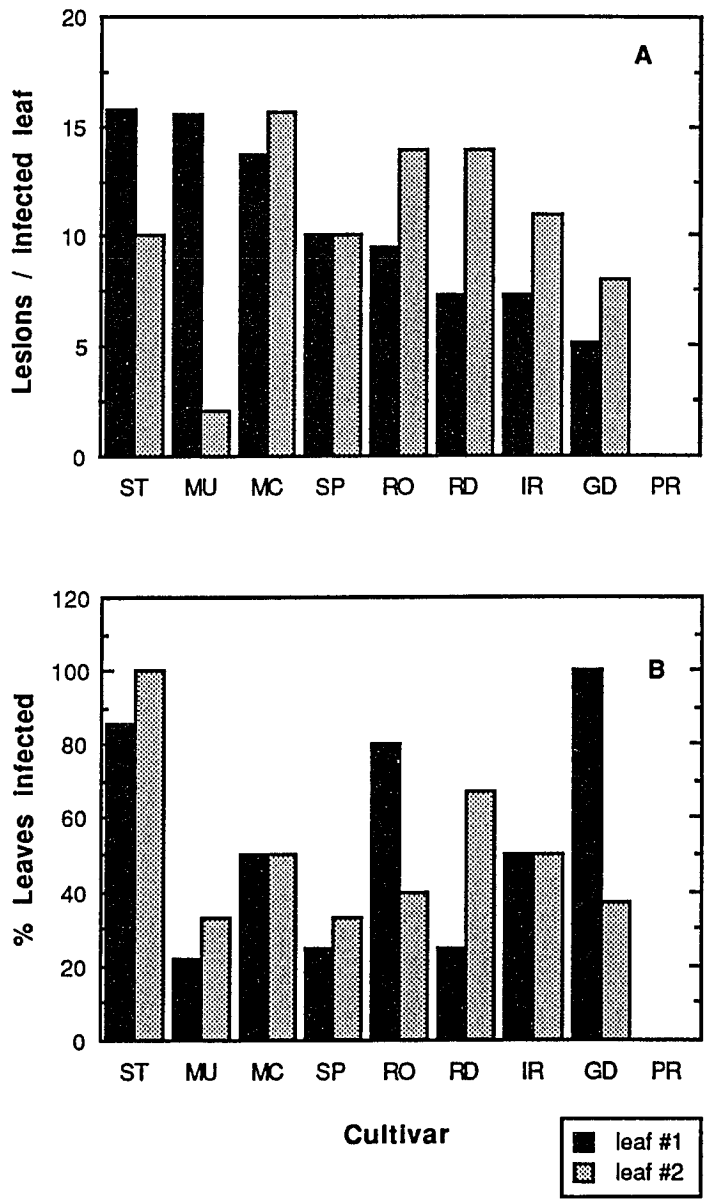


Fig. 40. The severity (A) and incidence (B) of apple scab caused by *Venturia inaequalis* on the youngest two leaves of nine apple cultivars in plastic pots in 1990. ST = 'Stayman,' MU = 'Mutsu,' MC = 'McIntosh,' SP = 'Spartan,' RO = 'Rome,' RD = 'Red Delicious,' IR = 'Ida Red,' GD = 'Golden Delicious,' and PR = 'Paula Red.'

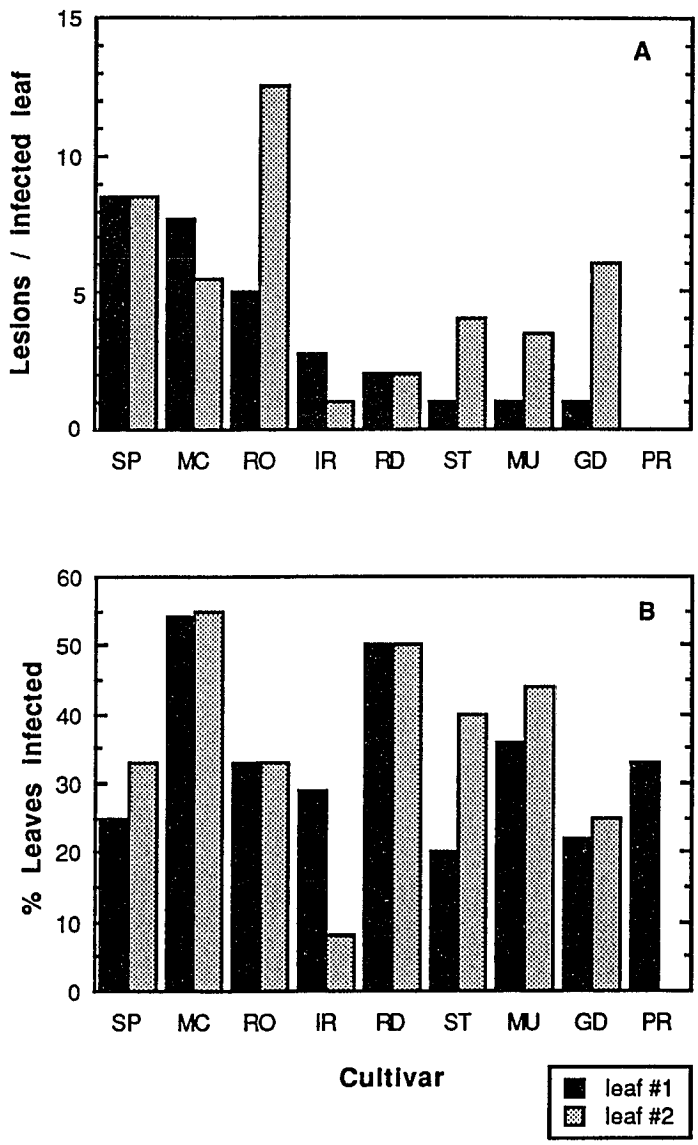


Fig. 41. The severity (A) and incidence (B) of apple scab caused by *Venturia inaequalis* on the youngest two leaves of nine apple cultivars in plastic pots in 1991. SP = 'Spartan,' MC = 'McIntosh,' RO = 'Rome,' IR = 'Ida Red,' RD = 'Red Delicious,' ST = 'Stayman,' MU = 'Mutsu,' GD = 'Golden Delicious,' and PR = 'Paula Red.'

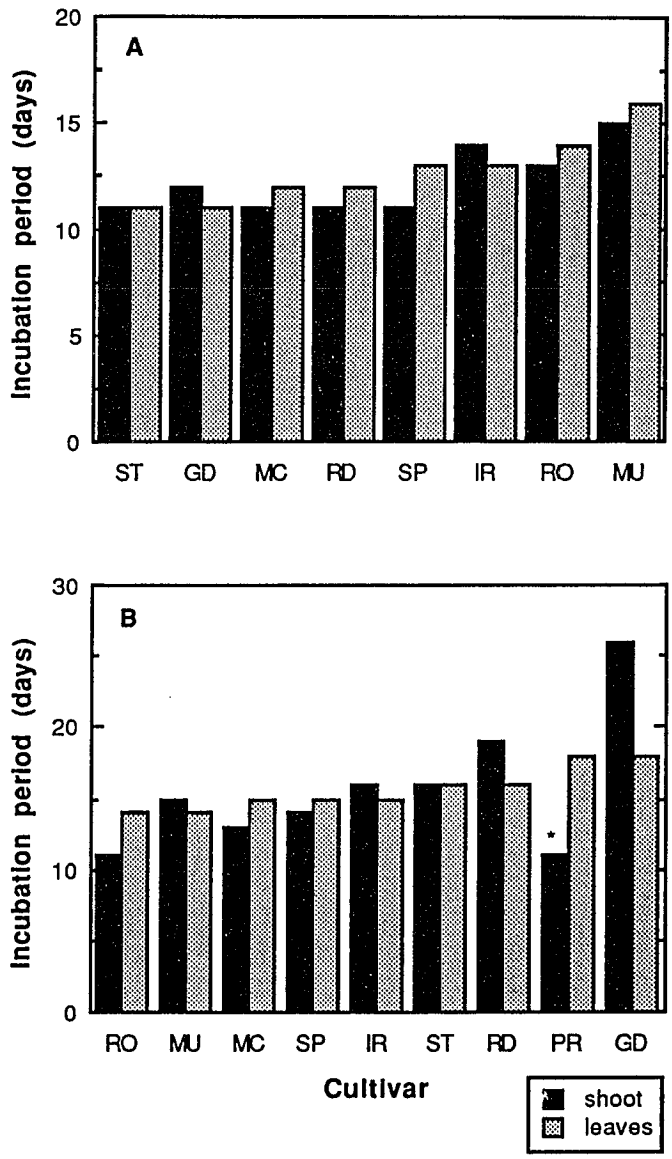


Fig. 42. The number of days between inoculation with *Venturia inaequalis* and the first appearance of sporulating lesions (incubation period) on extension shoots and leaves of nine apple cultivars in plastic pots in 1990 (**A**) and 1991 (**B**). ST = 'Stayman,' MC = 'McIntosh,' SP = 'Spartan,' RD = 'Red Delicious,' GD = 'Golden Delicious,' RO = 'Rome,' IR = 'Ida Red,' MU = 'Mutsu,' and PR = 'Paula Red.' * Data from a single infected leaf.

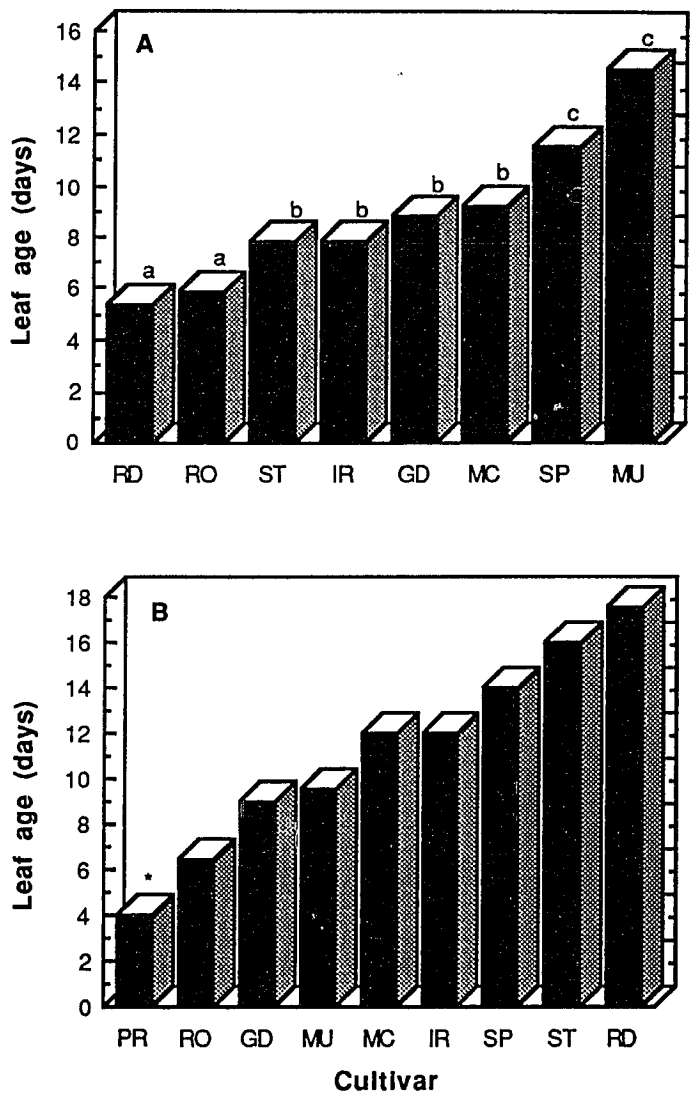


Fig. 43. The mean age of leaves, at the time of inoculation with *Venturia inaequalis* , that developed scab lesions in 1990 (A) and 1991 (B). Means labeled by the same letter do not differ significantly (P = 0.10). MC = 'McIntosh,' RD = 'Red Delicious,' MU = 'Mutsu,' SP = 'Spartan,' ST = 'Stayman,' GD = 'Golden Delicious,' IR = 'Ida Red,' RO = 'Rome,' and PR = 'Paula Red.' * Data from a single infected leaf.

Table 8. The relationship between apple scab severity[w] and incubation period (IPL)[x], leaf number[y] and leaf age[z], and the relationship between incubation period and leaf number and leaf age.

	Smith				Potted trees			
	1990		1991		1990		1991	
Regression	R^2	slope	R^2	slope	R^2	slope	R^2	slope
IPL v severity	0.10	-0.44	0.27	-1.23	0.30	-1.72	0.13	-0.15
leaf # v severity	0.01	-2.10	0.19	-11.2	0.06	-2.04	0.003	0.10
leaf # v IPL	-0.02	-0.05	0.49	6.98				
leaf age v severity					0.08	-0.50	0.05	-0.18
leaf age v IPL					0.12	0.20	0.01	-0.02

[w] Severity is the number of lesions/infected leaf.

[x] The incubation period (IPL) is the period (in days) from inoculation to the time when the first sporulating lesion became visible.

[y] Leaf number corresponds to the position (age) of the leaves on the extension shoot at the time of inoculation.

[z] Leaf age is the age of the leaf at the time of inoculation, measured in days after unfurling.

Discussion

Disease Assessments

Disease incidence and severity on extension shoot and cluster leaves, on sepals, and on fruit differed among the cultivars in 1988, 1989, and in 1990. The relative ranking of the resistance of the cultivars based on scab incidence varied from year to year. These findings generally support the data from several studies (5, 57, 63, 96, 119), but there are shortcomings with these studies. The data reported by Aldwinkle (5) was subjective. The rating of apple cultivars for scab resistance was based on responses to a questionnaire sent to growers and specialists in three geographic areas of the United States, and the mean resistance rating for some cultivars was based on as few as one response to the questionnaire. The results reported by Hough (57) and Olivier (96) were based on quantitative data, but a limited number of cultivars were compared. The incidence and severity of scab on thirty cultivars was compared by Szkolnik (120) in field and greenhouse studies, but the data from the field studies were inconclusive; therefore, only the data from the greenhouse studies were used for comparing cultivar susceptibility. The present study is the first to compare and quantify disease incidence and severity and disease progression on apple cultivars under field conditions.

The rate of scab buildup was significantly greater ($P = 0.05$) on 'McIntosh' and 'Rome' in 1989 and in 1990, but in 1988 the rate of scab buildup was greater on 'Stayman' than on any of the remaining eight cultivars. The greater rate at which scab increased on 'Stayman' in 1988 may have resulted from exposure to the extremely high inoculum dose supplied from heavily scabbed 'McIntosh' leaves placed in the orchard in Nov 1987. The absence of severe infection periods during the time of rapid shoot growth (emergence of new leaves) most likely accounted for the occasional decrease in disease incidence recorded for several cultivars in 1989 (Fig. 9). The slower rate of disease development (based on

foliar incidence) in 1989 compared to 1988 and 1990 was due to a decrease in the number of secondary infection periods (8 in 1989; and 13 in 1988 and in 1990). The foliage on 'Spartan' was noticeably bronzed due to mite feeding, so the decrease in foliar scab on 'Spartan' that began on 3 Jul was most likely the result of extremely high mite pressure (102).

The cultivars can be grouped into three resistance categories based on the incidence of foliar scab for the three years of the study: 'McIntosh' and 'Rome' were least resistant (> 20 % foliar scab), 'Stayman,' 'Mutsu,' 'Spartan,' and 'Golden Delicious' were moderately resistant (> 5 % but < 20 % foliar scab), and 'Delicious,' 'Paula Red,' and 'Ida Red' were highly resistant (< 5 % foliar scab). The relative ranking of the four cultivars in the moderately resistant category varied from year to year, but all four cultivars remained in that category except for 'Stayman' in 1988. The relative resistance of the cultivars reported in the present study is not in agreement with two other studies (5, 120), particularly regarding 'Paula Red' which was rated highly susceptible in both studies. Hough (57) provided two possible explanations to account for the different rankings of cultivar susceptibilities in different studies: (i) the cultivars may have been clones of genetically different seedlings or (ii) the local population (strain) of *V. inaequalis* may have differed among the studies. Although it is possible that the cultivars were different clones, given that cultivated apple cultivars are vegetatively propagated (grafted) by the various nurseries and that different cultivars from different nurseries are distinguished by name (often a registered trade mark), it is more likely that the local populations of *V. inaequalis* differed among the studies. Also, as discussed previously, the ratings reported by Szkolnik (120) and Aldwinkle (5) were based on greenhouse or subjective data.

The phenological studies included in the present research provided evidence that 26 to 40 % of the fruit buds were at green-tip during either of two primary infection periods in 1990. It was also determined that the first tissues exposed at green-tip were the sepals and the tips of the cluster leaves, and this agreed with the observation made by Keitt and Jones

(68) and Johnstone (63). The early establishment of fruit infections (sepals) on 'Spartan' was correlated ($R^2 = 0.68$) with 'Spartan's significantly greater ($P = 0.05$) incidence of infected fruit relative to the other cultivars. The extreme cracking and deformation of infected 'Spartan' fruit (Fig. 20) also suggests that infection occurred early (63). Based on the results from 1988, 1989, and 1990, it appears that the fruit become infected very early in their development, often at green-tip. In all cultivars , except 'McIntosh' and 'Rome,' there was very little increase in incidence of infected fruit after the first disease assessment (Figs. 15-17), suggesting that the fruit develop a high resistance to *V. inaequalis* within two weeks after fruit-set. The fruit of 'McIntosh' and 'Rome' do not appear to develop resistance to *V. inaequalis* as shown by the continued increase in the incidence of scabbed fruit throughout the growing season, provided conditions were favorable for infection.

It was important to determine if the cultivars differed in their relative resistance to scab. If the cultivars had not differed in relative resistance to scab, then further studies on the components of partial resistance would not have been warranted.

Leaf Area and Phenology

Leaf Area. The area (cm^2) of susceptible green tissue exposed to apple scab inoculum was compared among the cultivars at four stages of fruit bud growth and for each of the five youngest extension shoot leaves. The area of susceptible tissue differed among the cultivars at all four fruit bud growth stages and for all five extension shoot leaves. The differences in area of susceptible tissue exposed were due to the inherent characteristics of the apple cultivars. The fruit buds of 'Ida Red' and 'Mutsu' were generally wider in diameter than those of the other cultivars. The greater width of the bud would allow a greater area of susceptible tissue to emerge out of the wider bud apex. The differences in area were less pronounced at 1.27 cm green-tip because the buds were being forced open by the emerging tissues. The size and number of leaves per cluster determined the area of tissue exposed at open-cluster. Individual cluster leaves on 'Rome,' 'Mutsu', and 'Ida

Red' were larger and usually more numerous per cluster when compared to the cluster leaves on the other cultivars.

Extension shoot leaves older than the fifth unfurled leaf were not included in the leaf area comparisons because it was determined during the study on ontogenic resistance and latent period that these leaves rarely developed apple scab lesions. The area of the first five leaves of each cultivar represents the area of leaf tissue susceptible to infection by *V. inaequalis* between the time the first shoot leaf unfurls and terminal bud-set (Table 2).

A simple measurement of leaf length and leaf width (at the widest point) can provide an accurate estimate of leaf area. Boynton and Harris, reported that leaf area on 'McIntosh' could be determined by multiplying the product of leaf width and length by a predetermined factor. The "predetermined factor" equaled the slope from a regression of leaf area against the product of leaf width and length. A similar relationship between leaf area and leaf width and length was noted on 'McIntosh' and 'Rome Beauty' by MacHardy (unpublished data). A strong correlation was obtained when actual leaf area was regressed against the product of leaf length and width for each cultivar ($R^2 \geq 0.98$ for all cultivars), or leaf width ($R^2 = 0.90$ for all cultivars). Regression analysis may not always be practical or possible however, so the leaf area index number (LAI = actual leaf area ÷ the product of leaf length and leaf width) was calculated for each cultivar. Once the LAI has been determined for a cultivar, only the leaf length and width (mm) need to be measured, and a simple calculation would provide leaf area. This technique is particularly useful where quick, non-destructive sampling is required and where a portable leaf area meter is not available.

Additional measurements of the area of tissue exposed at tight-cluster and when 1 to 2, and 3 to 5 cluster leaves have expanded would be useful. This additional information, combined with the measurements from the four fruit bud stages and the five youngest leaves would provide a full range of the area of susceptible tissue from green-tip to almost full leaf expansion. This information could be useful in predictive models for apple scab

development. The area of susceptible exposed to infection at any one time does not, when taken alone, appear to influence disease

Phenology. The rate of phenological development of woody plants is determined mainly by the temperature just prior to bud break and during the growing season (97, 109). Pack (97) reported that the phenological development of 15 species of trees and shrubs was related to the cumulated hours of warm (> 15.6 C) and cold (< 4.4 C) temperatures. The phenological development of fruiting buds on six apple cultivars was strongly correlated ($R^2 = 0.98$) with the natural logarithm of cumulative growing degree hours (base = 5.5 C) (109). Temperature may determine the rate of bud development, but the effect of temperature would have been the same for all cultivars in the present study since they were all located within a single 0.5 ha block.

The development of apple fruit buds was divided into 16 sequential phenophases similar to those described by Seem and Szkolnik (109). This phenophase system (Table 1) allowed subtle shifts in development to be more easily identified than with the standard 7-9 bud stages included in many apple spray guides. However, the sample size of 30 buds was too small for statistical comparison of phenological development among the cultivars, based on the 16 phenophases. For statistical analysis, the 16 phenophases were grouped into 6 "phenology groups" based on the overlap of phenophases observed in the field (Figs. 29-31), and on bud stages that had been measured for leaf area (Figs. 26-28).

Phenological development among the nine apple cultivars was compared for each observation date. The cultivars differed in their stage of development on all observation dates except the final observation date each year when all cultivars were at fruit-set. Comparisons of bud development between the three years were not made, but several general trends were noted: (i) fruit buds on 'Ida Red' had the most rapid development up to bloom, (ii) buds on 'Rome' were the slowest to develop, (iii) buds on 'Delicious' developed slightly slower than on other cultivars through the first 4 bud stages, (iv) fruit buds on 'Mutsu' developed more quickly than on other cultivars between 1.27 cm and

open-cluster, and (v) all cultivars reached petal-fall or fruit-set within the same week. Seem and Szkolnik (109) also noted differences in phenological development among six apple cultivars in their study, but the differences were not analyzed statistically.

There was no consistent relationship between phenological development and the severity or incidence of apple scab among the cultivars. The rapid development of fruit buds on 'Ida Red' compared to the other cultivars may account for the low incidence of scab development on 'Ida Red.' It is possible that the rapid rate of bud development allowed exposed tissues to become resistant before key primary scab infection periods occurred, and, thus to "escape" infection (100). If 'Ida Red' had "escaped" all primary infection periods, no secondary scab would have developed. The slow rate at which fruiting buds developed on 'Rome' concomitant with consistently high levels of scab seems to support this idea. The delay in bud development could possibly have allowed the tissues to remain susceptible for prolonged periods of time, particularly during the periods of peak ascospore productivity. This apparent relationship (rapid development = less disease, slow bud development = more disease) was not consistent, however. 'McIntosh' was second only to 'Ida Red' in the rate of fruit bud development, yet was consistently one of the most severely infected cultivars. The rate of bud development alone does not appear to account for the differences in scab severity or incidence among the cultivars.

The rate of growth of the extension shoots did not differ among the cultivars (Fig. 32). As a result, the number of "new" susceptible leaves that emerged on a shoot per week was approximately the same for each cultivar. This may help to explain why the number of susceptible leaves/shoot was similar for each cultivar. The cessation of extension shoot growth (terminal bud-set) was recorded earliest on 'Paula Red' (20 Jul) and latest on 'Stayman' (31 Aug) in 1990. The differences in the time of bud-set among the cultivars was less than those reported by Olivier and Lespinasse (95). Several cultivars in their study produced new extension shoot leaves up to six months after bud-break while others set terminal bud in three months. They observed greater levels of disease development on

cultivars that had longer periods of extension shoot growth, but their data were not analyzed statistically. Similar trends were noted in the present study, but the relationship between the time of terminal bud-set and the severity or incidence of apple scab varied among the nine cultivars. The period of shoot growth ranged from 3 to 4 months. The early bud-set and lack of scab development on 'Paula Red' supported the findings of Olivier and Lespinasse. The period of shoot growth was equal for 'Mutsu' and 'Ida Red,' yet disease incidence and severity was significantly greater on 'Mutsu' compared to 'Ida Red.' The period or rate of shoot growth alone did not appear to account for the differences in scab development among the cultivars.

Conidia

The variation in spore production on infected tissues among cultivars is an important component of partial resistance (127), and several studies have investigated conidium production by *V. inaequalis* on apple scab lesions (52, 92, 95, 120), but none has compared conidium productivity on the same scab lesions on several cultivars over the period a lesion remained infectious. A simple, non-destructive sampling method was used that allowed the same lesion to be sampled over a period of several weeks.

Relative conidium production differed significantly ($P = 0.10$) among the cultivars in 1989 and in 1990 (Fig. 33), and this supports results reported by Szkolnik (120), Hickey (52), and Olivier and Lespinasse (95). Similar differences in spore productivity were reported for several wheat cultivars infected with leaf rust, caused by *Puccinia hordei* (90, 99). With all these studies, however, spore productivity was obtained by destructive sampling at a single collection date. Until the present study, there has been no information on the rate of conidium production by *V. inaequalis* .

The rate of conidium production did not differ significantly among the cultivars in 1989 or in 1990. This is not surprising as the lesions selected for sampling were fairly uniform in age (1 - 3 days), and all cultivars were exposed to the same relative humidity and temperature. Once conidia of *V. inaequalis* rupture the cuticle of an apple leaf,

temperature and relative humidity are known to exert a great influence on conidium production, but the influence of cultivar on conidium production has not been investigated (116, 117). In the present study, the only variable was cultivar, and there was no evidence indicating that cultivar influenced the rate of conidium production. Lesion size did not differ significantly among the cultivars, indicating that once a leaf was infected by *V. inaequalis*, the growth of the pathogen in the leaf was not influenced by cultivar.

The length of the infectious period did differ among the cultivars in 1989 and 1990. The mean infectious period on 'Golden Delicious' lesions (56 days in 1989) was significantly longer (P = 0.10) than the infectious period of the other cultivars and much longer than the 30 - 36 day infectious period reported by Nusbaum and Keitt (92). The longer infectious period recorded for all cultivars in 1990 (Table 5), was most likely due to the greater number of rain events in 1990 which may have provided moisture conditions more favorable for conidium production. There were no differences among the cultivars in the number of days required to reach 90 % conidium production. The 23 - 32 days required to reach 90 % conidium production was similar in length to the infectious period reported by Nusbaum and Keitt (92).

The conidia that are produced early in a scab epidemic have a greater effect on the development of an epidemic than those produced later (127, 141). The cumulative percent total conidia differed significantly among the cultivars on day 0 in 1989 and in 1990 (Fig. 36). Lesions on 'Spartan' and 'Rome' produced significantly more (P = 0.10) conidia on day 0 than the lesions on the other five cultivars in 1989 and in 1990, and this may have accounted for the greater buildup of scab on these cultivars earlier in the season in 1989 and 1990 (Figs. 10 &11, disease assessments).

Ontogenic Resistance and Latent Period

Incubation period, an integral component of partial resistance (101, 127), is defined as the elapsed time between inoculation and the appearance of disease symptoms. With apple scab, termination of the incubation period signals the onset of conidium production.

Ontogenic resistance, inherent to all apple cultivars, is generally regarded as increased resistance to *V. inaequalis* concomitant with an increase in leaf age.

The incubation period did not differ among the cultivars in the Smith block in 1990 or in 1991 (Table 6). In 1991, the incubation period among the cultivars was very narrow, i.e. 14-17 days, but in 1990 ranged from 16 days on 'McIntosh' to 23 days on 'Paula Red.' However, only three leaves were infected in 'McIntosh' and 'Paula Red,' and the variance among the three 'Paula Red' leaves was wide which accounted for the lack of significance in incubation period between the two cultivars.

Several investigations on apple scab have reported a strong relationship between incubation period and leaf age (46, 48, 92). In the present study, incubation period was related more closely to leaf age than to partial resistance. The ranking of cultivars based on length of the incubation period is not consistent with the ranking of the cultivars based on scab severity (Table 7). Incubation period was poorly correlated with leaf age ($R^2 = 0.20$). Gessler and Stumm (48) reported that the formation of primary stromata of *V. inaequalis* in apple leaves was affected by the age of the leaf but not by cultivar when primary stroma development was compared in susceptible 'Golden Delicious' and resistant 'Liberty' leaves. The longer incubation period on 'Mutsu' leaves then, was not surprising since the leaves on 'Mutsu' were the oldest at the time of inoculation. Studies on wheat infected with *Puccinia hordei* (99, 90), and lettuce infected with *Bremia lactucae* (32) however, showed that incubation period was strongly correlated with disease resistance. How can the effect of cultivar on incubation period be addressed experimentally for *V. inaequalis* on apples? The experiment would need to include a much larger sample of leaves of known age than were used in the present study to ensure that a sufficient number of leaves would be infected to allow statistical analysis.

The small number of infected leaves made it difficult to accurately assess ontogenic resistance in these experiments. But, several general conclusions can be gleaned from the data. In the Smith block, few leaves older than the fourth leaf from the shoot tip developed

scab, and usually only leaves 1 - 3 were infected. The results agree with Moore (88) and Johnstone (63). Johnstone (63) inoculated shoots with five leaves and found that although all five leaves developed scab lesions, only the youngest three leaves developed extensive scab. Moore (88) reported that leaves further than the fifth leaf from the shoot tip were resistant to scab.

In the potted trees, few leaves older than the third leaf were infected, and rarely did leaves older than 12 days at the time of inoculation develop lesions. The incidence of apple scab on leaves younger than 12 days at inoculation was 61 % compared with an incidence of only 15 % on leaves older than 12 days at inoculation. Other studies (46, 107) have also reported that leaves older than 12 days at inoculation rarely developed scab lesions. In one of these studies (107) the leaves were nearly fully expanded 12 days after unfurling, and this is in agreement with the present study.

From the results of the present study, it does not appear that the time required for *V. inaequalis* to infect its host and cause a lesion to develop was influenced by cultivar. However, the age of a leaf when infected did influence the time required to produce a lesion, but the influence of age was independent of cultivar.

FINAL STATEMENTS

Summary of the relationships between cultivar and several components believed to contribute to partial resistance

The area of exposed tissue differed among the cultivars at each growth stage measured. The area (cm^2) of exposed tissue combined with phenological development, could be used to estimate area of susceptible tissue on any day during the growing season. The phenological growth stages overlap, and this is why differences in the area of exposed tissue were not as great as first thought. The youngest five extension shoot leaves of each cultivar represent the area of susceptible shoot tissue between the time the first leaf unfurled and terminal bud-set, and the area of the youngest five leaves differed among the cultivars. It is possible that a larger area of exposed tissue would increase the likelihood of contact between the host and the inoculum, but, the experiments were not designed to look at that relationship.

The phenological development of apple fruit buds provides information on the time that a particular bud stage occurs relative to apple scab infection periods. The rate of extension shoot growth (leaves emerging/week) did not differ among the cultivars. Therefore, the number of days required for a furled leaf to reach full leaf expansion (to become resistant) is the same for all cultivars, and this interval was approximately twelve days under the conditions of the study. One of the most promising applications of the phenological and leaf area data is its utilization in apple scab predictive models because this information has not been available.

The mean number of ascospores produced on a fertile lesion was influenced by cultivar, but the rate at which the ascospores matured was not. The rate of maturation was most likely determined by temperature (i.e., accumulated degree-days).

The relative conidium productivity/lesion was influenced by cultivar. In particular, the production of conidia for approximately the first ten days after a lesion appeared varied considerably among the cultivars. An early buildup of scab was noted in cultivars with lesions that had high conidium productivity during the first ten days after lesion appearance. The length of the infectious period differed among the cultivars. A long infectious period was correlated with greater conidium productivity.

The incubation period was not strongly influenced by cultivar, but it did appear to be correlated with leaf age. On small trees, the greater the age of the leaf, measured by days after unfurling, the longer the incubation period.

An ontogenic resistance mechanism was apparently operational by the 12th day after a leaf had unfurled, regardless of cultivar.

Regression analysis indicated that foliar scab incidence and severity were poorly correlated ($R^2 = 0.30$) with nearly all components of partial resistance investigated. Exceptions were a relationship between foliar scab incidence and severity each, or between years (i.e., 1989 foliar scab incidence vs 1990 foliar scab incidence, $R^2 = 0.91$, and 1990 foliar scab incidence vs 1990 foliar scab severity, $R^2 = 0.68$). Why were the correlations so poor?

(1) The variability of the data was great. The sample size was too small (i.e., an insufficient number of leaves developed scab in the ontogenic and incubation period study).

(2) For many components, there was a noticeable trend in the data, but that is all that can be said about it without statistical evidence.

Conclusions Regarding the Resistance of the Nine Apple Cultivars to Scab

Although 'Golden Delicious' ranked third in severity among the nine cultivars when disease and inoculum measurements were considered and averaged (Table 9), the actual susceptibility of based on lesion incidence and severity only, lowered 'Golden Delicious' to sixth relative to the other cultivars (Table 10). Considering the high amount of primary and secondary inoculum produced on lesions on 'Golden Delicious,' the development of scab was surprisingly low compared to other cultivars. It appears that although 'Golden Delicious' does not become infected easily, once infected however, the pathogen survives and reproduces very well. Thus, it appears that 'Golden Delicious' is moderately resistant, even under fairly high inoculum dose.

'McIntosh' and 'Rome' are the least resistant cultivars, based on the incidence and severity of foliar scab (Tables 9 & 10).

'Stayman' susceptibility was highly variable, but other than in 1988 when it ranked first among the cultivars for disease assessments (Table 9), it was moderately resistant.

'Mutsu' and 'Spartan' were moderately resistant, with 'Mutsu' being slightly less resistant than 'Spartan' (Tables 9 & 10).

'Delicious' was moderately resistant when all components for disease and inoculum were considered, but it appeared to be slightly more resistant than 'Spartan' or 'Mutsu' (Tables 9 & 10).

'Ida Red' and 'Paula Red' were highly resistant to the local population of *V. Inaequalis.*

Table 9. The relative ranking of nine apple cultivars infected with *Venturia inaequalis* based on foliar disease incidence and severity, ascospore productivity, and conidium productivity.

	Foliar scab						ascospore productivity		conidium productivity	
	1988		1989		1990					
Cultivar	incidence	severity	incidence	severity	incidence	severity	1990	1991	1989	1990
McIntosh	2 (0.45)[x]	3 (0.56)	1 (1.00)	3 (0.81)	1 (1.00)	2 (0.96)	2 (0.77)	4 (0.21)	5 (0.32)	5 (0.36)
Rome	6 (0.06)	2 (0.76)	2 (0.48)	1 (1.00)	2 (0.70)	1 (1.00)	1 (1.00)	1 (1.00)	3 (0.60)	2 (0.58)
Stayman	1 (1.00)	1 (1.00)	3 (0.22)	4 (0.07)	6 (0.03)	6 (0.51)	7 (0.31)	—[1]	4 (0.50)	4 (0.37)
Mutsu	4 (0.18)	4 (0.49)	5 (0.12)	2 (0.85)	3 (0.11)	5 (0.51	6 (0.35)	3 (0.39)	2 (0.66)	6 (0.29)
Spartan	5 (0.11)	7 (0.27)	4 (0.17)	6 (0.52)	4 (0.11)	4 (0.55)	3 (0.70)	6 (0.06)[y]	6 (0.22)	3 (0.50)
Golden Del	8 (0.01)	8 (0.13)	4 (0.17)	5 (0.68)	5 (0.06)	5 (0.60)	4 (0.66)	2 (0.88)	1 (1.00)	1 (1.00)
Delicious	6 (0.06)	5 (0.42)	6 (0.06)	7 (0.39)	8 (.002)[z]	8 (0.19)[z]	5 (0.50)	5 (0.12)[y]	7 (0.18)	7 (0.11)
Ida Red	7 (0.06)	6 (0.33)	7 (0.00)	8 (0.00)	9 (0.00)	9 (0.00)	—	—	—	—
Paula Red	9 (0.00)	9 (0.00)	7 (0.00)	8 (0.00)	7 (.003)[z]	7 (0.38)[z]	—	—	—	—

[x] First number is the relative numerical ranking of each cultivar; the number in parenthesis is the proportion of the maximum value recorded for the component.

[y] Ascospores were difficult to accurately count because the slides were heavily contaminated with *Leptospharia sp*; the actual number of ascospores may have been higher than the recorded number.

[z] Based on a single infected leaf.

[1] No lesions developed.

Table 10. The mean relative rankings for disease level,and inoculum productivity on nine apple cultivars infected with *Venturia inaequalis*.

Disease level[t]				Inoculum productivity							
				Conidia[u]				Ascospores[v]			
Cultivar	Proportion of max[w]	% reduction[x]	Rank[y]	Cultivar	Proportion of max	% reduction	Rank	Cultivar	Proportion of max	% reduction	Rank
MC[z]	1.00	0	2.0	GD	1.00	0	1.0	RO	1.0	0	1.0
RO	0.92	8	1.8	RO	0.59	41	2.5	GD	0.96	4	3.0
ST	0.59	41	3.3	MU	0.47	52	4.0	MC	0.61	39	3.0
MU	0.47	53	3.8	ST	0.44	56	4.0	SP	0.47	52	4.5
SP	0.36	64	5.0	SP	0.36	64	4.5	MU	0.46	54	4.5
GD	0.34	65	5.5	MC	0.34	66	5.0	ST	0.39	61	7.0
RD	0.24	76	6.5	RD	0.14	85	7.0	RD	0.39	61	5.0
IR	0.08	92	7.5	IR	—[1]	—	8.0	IR	—	—	8.0
PR	0.08	92	7.7	PR	—	—	8.0	PR	—	—	8.0

[t] Means from assessments of scab incidence and severity in 1988, 1989, and 1990. Data derived from Table 9.

[u] Calculated from mean conidium productivity in 1989 and 1990.

[v] Calculated from mean ascospore productivity in 1990 and 1991.

[w] Cultivars are; MC = 'McIntosh,' RO = 'Rome,' ST = 'Stayman,' MU = 'Mutsu,' SP = 'Spartan,' GD = 'Golden Delicious,'RD = 'Delicious,' IR = 'Ida Red,' and PR = 'Paula Red.'

[x] Proportion of max = proportion of the greatest value recorded which was set at 100.

[y] Percent reduction compared to the maximum value calculated for the component.

[z] Relative numeral ranking of each cultivar based on mean calculated for each cultivar.

[1] No lesions developed.

LITERATURE CITED

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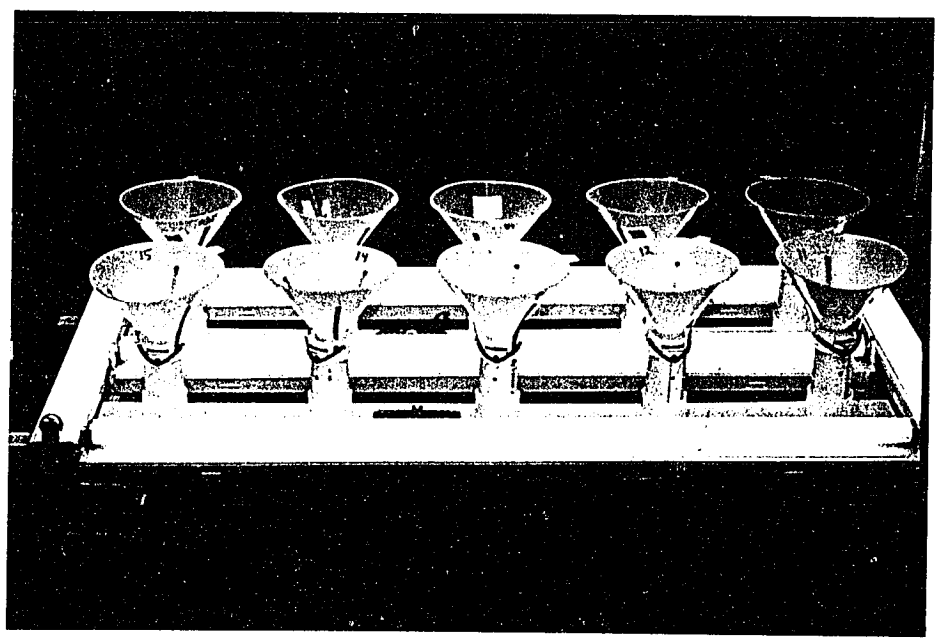
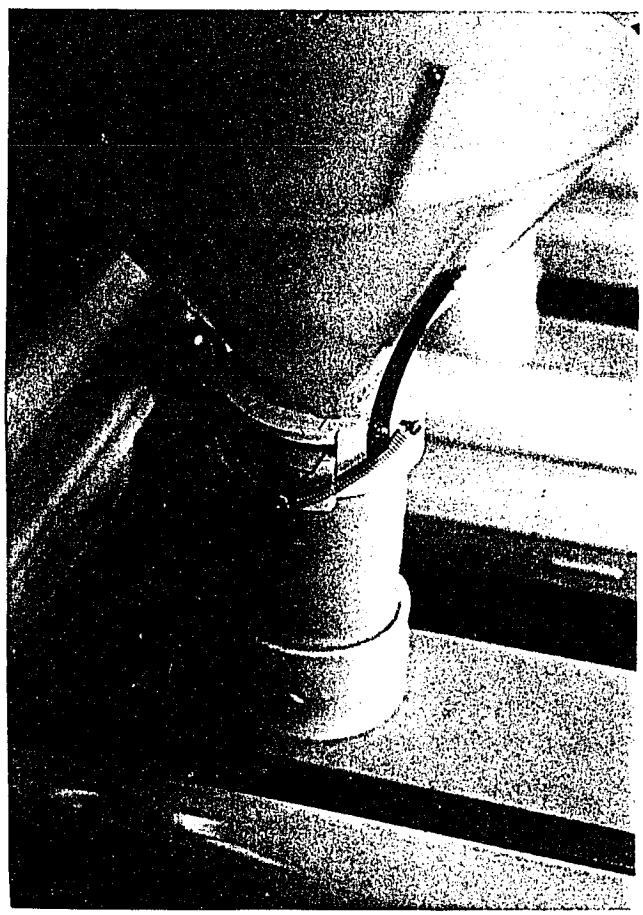
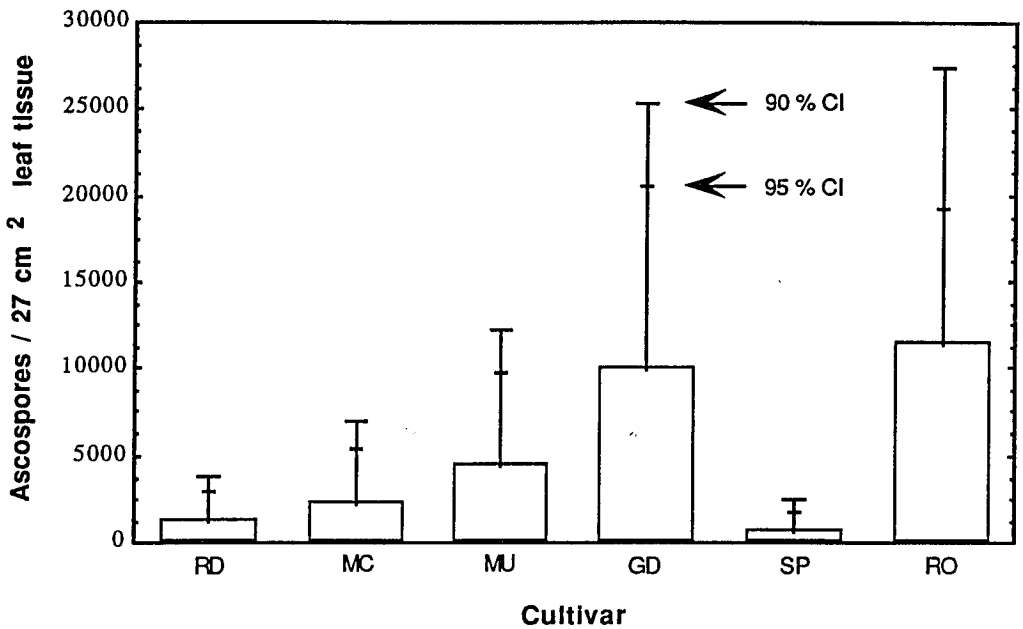
APPENDIX



Appendix Fig. 1. "Funnel-traps" used for ascospore productivity study in 1990 and 1991.



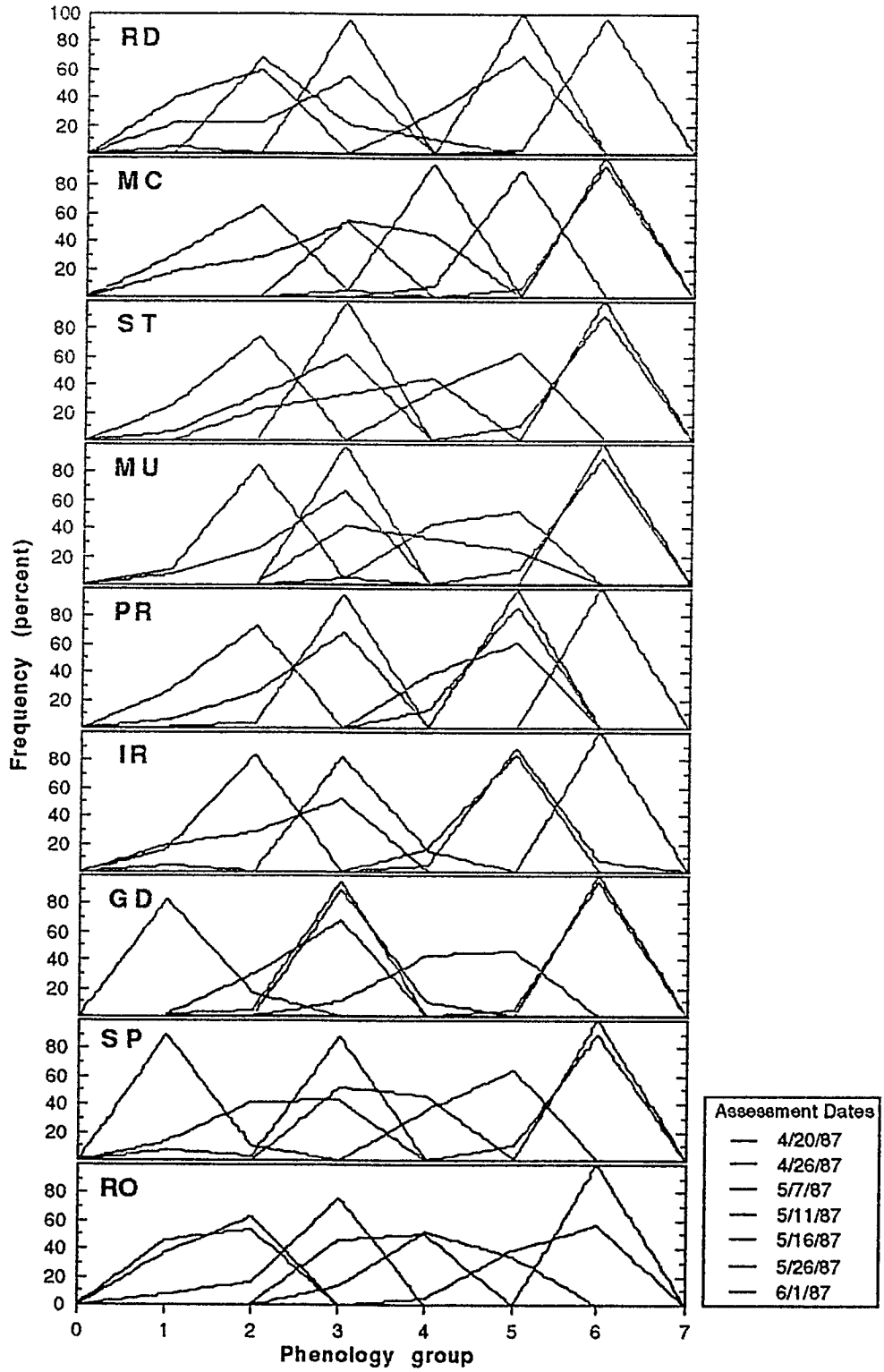
Appendix Fig. 2. Close-up of "Funnel-trap," showing the positioning of the collection slide at the base of the funnel.



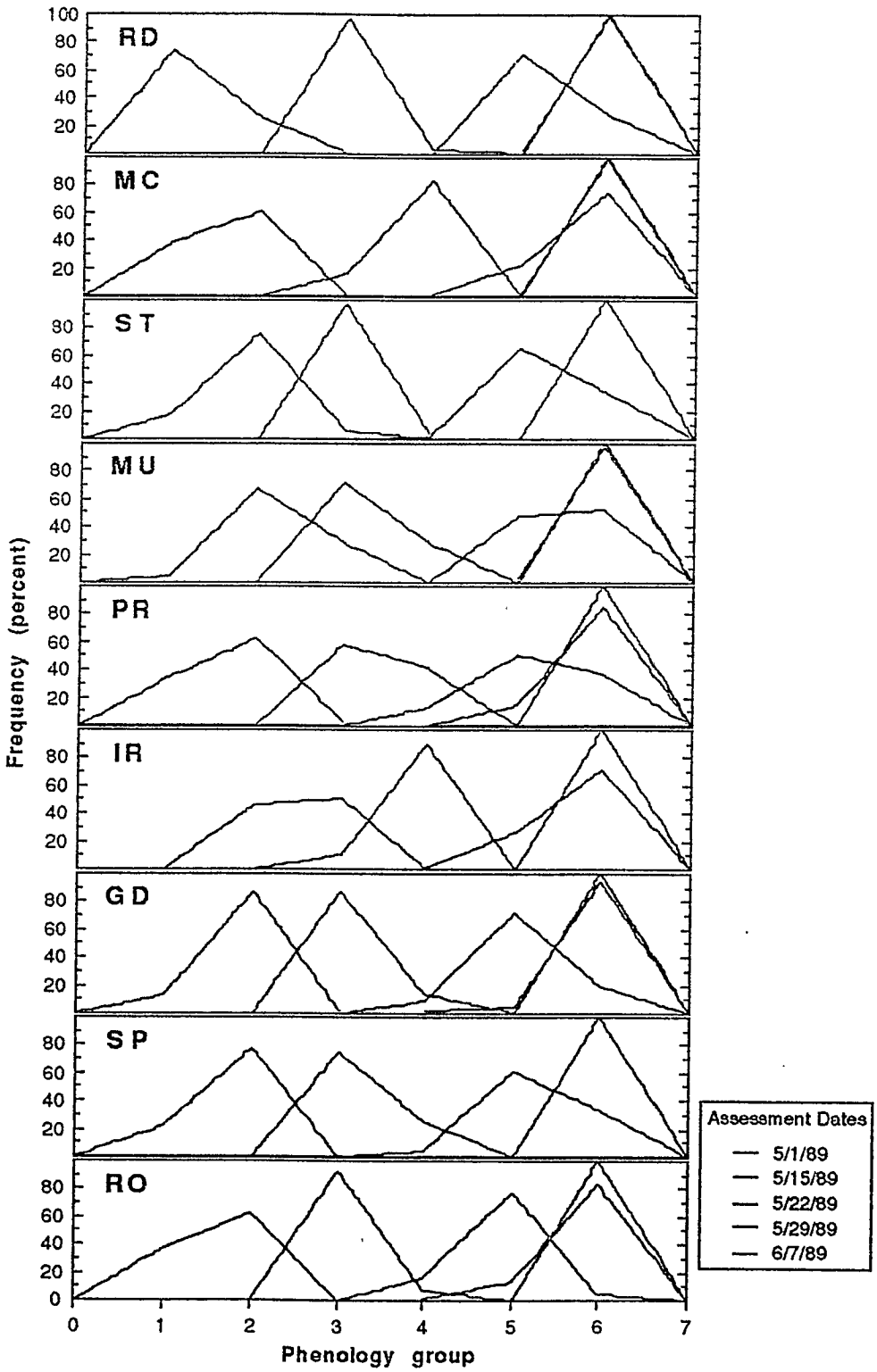
RD = Red Del	GD = Golden Del
MC = McIntosh	SP = Spartan
MU = Mutsu	RO = Rome

Appendix Fig. 3. Ascospore productivity on leaf discs infected with *Venturia inaequalis* in 1991. Bars represent the mean for each cultivar. Vertical lines represent the 90% and 95% confidence intervals (arrows) of the mean ascospore productivity for each cultivar.

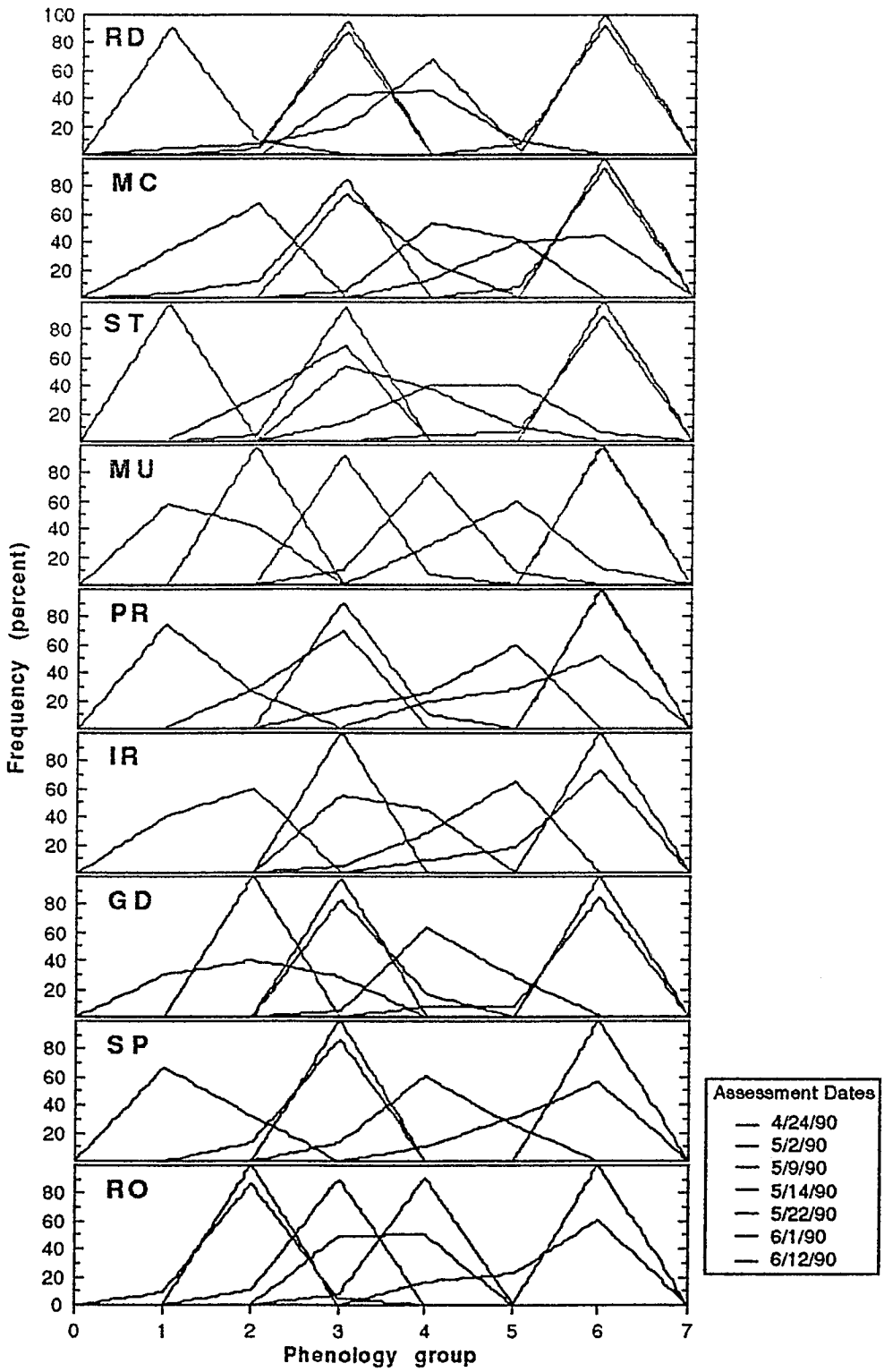
Appendix Fig. 4. The frequency of apple fruit bud "phenology groups" on nine apple cultivars on each sampling date in 1987. The six "phenology groups" were derived from 16 phenophases (growth stages) for the purpose of statistical analysis. RD = 'Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'

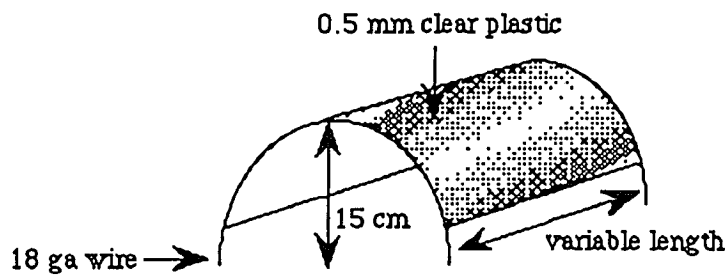


Appendix Fig. 5. The frequency of apple fruit bud "phenology groups" on nine apple cultivars on each sampling date in 1989. The six "phenology groups" were derived from 16 phenophases (growth stages) for the purpose of statistical analysis. RD = 'Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'



Appendix Fig. 6. The frequency of apple fruit bud "phenology groups" on nine apple cultivars on each sampling date in 1990. The six "phenology groups" were derived from 16 phenophases (growth stages) for the purpose of statistical analysis. RD = 'Delicious,' MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' IR = 'Ida Red,' GD = 'Golden Delicious,' SP = 'Spartan,' and RO = 'Rome.'





Appendix Fig. 7. Diagram of "poly -house" used to protect scab lesions from rainfall between collections of conidia of *Venturia inaequalis* .

Table 11. Slope coefficients and the 95 % confidence intervals of the slope, determined by regression analysis of data from 1990 ascospore productivity, 1989 conidium productivity, and 1988 and 1990 foliar lesion incidence for nine apple cultivars[x].

	1990 Ascospore Productivity		1989 Conidium Productivity		1988 Foliar Incidence		1990 Foliar Incidence	
Cultivar	Slope	95 % CI[y]	Slope	95 % CI	Slope	95 % CI	Slope	95 % CI
RD	0.006	.006-.008	8.73	4.0-13.4	0.48	0.22-0.73	0.001	0.00-0.002
MC	0.006	.006-.008	8.02	3.4-12.7	0.62	0.32-0.91	0.74	0.55-0.92
ST	0.006	.005-.007	7.84	3.2- 2.4	0.099	0.68-1.31	0.24	0.06-0.42
MU	0.007	.005-.008	6.4	2.4-10.3	0.49	0.16-0.82	0.38	0.23-0.53
PR	—[z]	—	—	—	- 0.08	-0.42-0.26	—	—
IR	—	—	—	—	0.03	-0.32-0.38	0.004	-0.01-0.02
GD	0.006	.005-.008	6.79	3.2-10.3	0.24	-0.32-0.80	0.33	0.13-0.52
SP	0.007	.005-.009	5.28	2.4- 8.2	0.21	-0.04-0.47	0.35	-0.02-0.72
RO	0.008	.006-.009	6.15	2.9- 9.4	1.01	0.47-1.56	0.67	0.32-1.02

[x] RD = 'Delicious,' 'MC = 'McIntosh,' ST = 'Stayman,' MU = 'Mutsu,' PR = 'Paula Red,' GD = 'Golden Delicious,' SP = 'Spartan,' RO = 'Rome.'

[y] CI = confidence interval.

[z] No lesions developed.