Detection of Variation in Virulence Toward Susceptible Apple Cultivars in Natural Populations of *Venturia inaequalis*

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ABSTRACT

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Isolates of *Venturia inaequalis* taken from primary lesions on seven apple cultivars (Golden Delicious, Idared, Maigold, Glockenapfel, Boskoop, Spartan, and James Grieve) were tested as mixtures or alone in cross-infection trials on graft trees of the seven cultivars. In general, isolates produced the most lesions on the cultivar genotypes from which they were isolated. None of the isolates produced the same infection type on all cultivars. Two cultivars, Boskoop and Glockenapfel, were the most resistant to most inocula but considerably less resistant to the isolates originating from them. Maigold and Golden Delicious were infected severely by all isolates. In general, specific pathogen genotypes showed specialization toward particular host genotypes. Monoconidial cultures were identified and distinguished by random amplified polymorphic DNA (RAPD) markers. In competition experiments among isolates from different cultivars inoculated on single hosts, there was strong selection against isolates not originating from the particular test cultivar. This was true even for the most susceptible apple cultivars. These results indicate the presence of differential resistance factors in susceptible cultivars that exert selection on natural *V. inaequalis* populations that vary in virulence. The implications of these observations for disease control are discussed.

Additional keywords: *Malus* × *domestica*, *PCR*, *scab*.

Apple cultivars are generally regarded as susceptible to scab caused by *Venturia inaequalis* (Cooke) Wint. The degree of susceptibility may vary between cultivars, but none are immune, except for new cultivars into which resistance from wild *Malus* species has been introgressed (6). In 1899, Aderhold (1) pointed out that the resistance of a single cultivar varies at different sites and, therefore, depends on the interaction between a specific cultivar and a specific isolate (or environment). More-detailed investigations (15,17–21,27) confirmed these observations and showed that a particular isolate more severely attacks the cultivar from which it originated.

Isolates of *V. inaequalis* originating from crabapples, analyzed genetically on *Malus* × *domestica* Borkh., had 19 genes conditioning virulence (5). Some of these interactions were indicative of a gene-for-gene relationship between pathogen and host (7,25) for fleck and lesion characters (5). Because the sexual stage provides the primary inoculum during each season in most regions, there is ample opportunity for the generation, annually, of many combinations of the alleles of these genes (5). As a consequence, there seems little point in classifying lines of *V. inaequalis* into races based only on pathogenic reactions. Nevertheless, to date, six races of *V. inaequalis* have been described (16,22,28) with *M. micromalus*, *M. atrosanguinea*, *M. baccata*, *M. b. jackii*, *M. zumi calocarpa*, R12740-7A, *M. floribunda* 821, and Antonovka as differential sources of resistance rather than cultivars of *M. domestica*. Isolates attacking *M. domestica* cultivars were classified as race 1.

Resistance-breeding programs are based on the introgression of major genes from wild *Malus* species, because it is assumed that no major resistance genes are present in *M. domestica*. The introgression of the resistance of *M. floribunda* 821 into a commercially acceptable cultivar took 60–80 yr (6). The use of existing differential resistances of common apple cultivars in breeding programs could reduce enormously the time it takes to breed new, more resistant, and commercially acceptable cultivars. Recent investigations showed that some older *M. domestica* cultivars may possess differential resistances against selected strains of *V. inaequalis* (23). Such differential resistances are of little value alone in individual cultivars that are grown in current monocultural systems. However, they may have an important place in sustainable production systems for cultivar mixtures or for pyramiding resistance in new cultivars (4,8).

We report on the specific interactions between a set of known and popular apple cultivars and natural field strains of the scab fungus and on selection exerted by the host on the pathogen population.

MATERIAL AND METHODS

The trials were carried out in two different years. During May 1992, 21 lesions were taken, seven from each cultivar—Boskoop, James Grieve, and Spartan. During May 1993, 60 lesions were collected, 10 from each of the five economically most important apple cultivars in Switzerland—Golden Delicious, Idared, Glockenapfel, Maigold, and Boskoop—as well as 10 from Spartan for a control group. These were from untreated trees planted in three orchards located throughout Switzerland (Wädenswil: 47° 13′ N, 8° 41′ E; Eschikon: 47° 27′ N, 8° 40′ E; and Chateauxneuf-Sion: 46° 13′ N, 7° 20′ E).

Isolation. Spores from a primary lesion (assumed to be caused by ascospores) were washed off with 5-μl sterile water drops and diluted to a concentration of 15–20 × 10^3 conidia per milliliter. One- to five-microliter drops were placed on agar (1.2% agar, 1.5% malt extract, and terramycin at 25 μg/ml), spread, and incubated for 24 h at 20 C. Under an inverted microscope, single conidia were transferred to a fresh agar plate and incubated at 20 C. The resulting cultures were named Boskoop 1–7 (1992) and 8–15 (1993), James Grieve 1–7 (1992), Spartan 1–7 (1992) and 8–14 (1993), Golden Delicious 1–7 (1993), Idared 1–10 (1993), Glockenapfel 1–6 (1993), and Maigold 1–11 (1993).

Mixed-population trials. Spores from the 21 lesions collected in 1992 were mixed in equal amounts to a final concentration of 15–20 × 10^4 conidia per milliliter, and the cultivars were inocu-

lated. After lesions appeared, the resulting conidia were collected, and each cultivar was reinoculated with its own inoculum. After three such asexual cycles, 50 monospore isolations were made from the sporulating lesions from each cultivar (described above).

In 1993, conidia from the 10 lesions of one particular cultivar were mixed in equal amounts to a final concentration of 15–20 $\times 10^4$ conidia per milliliter. The cultivars were inoculated with their own inoculum, and after lesions appeared, the conidia were washed off. After this asexual cycle (one propagation cycle), 50 monosporic cultures were made from the sporulating lesions from each cultivar (described above).

Cross-infection. In 1992, cross-infections were made with inoculum collected during July from each of the cultivars Boskoop, James Grieve, and Spartan at 15–20 $\times 10^4$ conidia per milliliter. In 1993, conidia, originating from the same lesions as used for the 50 monosporic cultures, were inoculated onto each of the cultivars (1 yr old on rootstock M26). The incubation conditions were 19 C and 100% relative humidity for 24 h. The macroscopic symptoms were classified after the 17th day of incubation: 4 = obvious and strongly sporulating lesions; 3 = well sporulating but weak chlorotic lesions; 2 = small, chlorotic or weakly sporulating flecks; 1 = small, chlorotic flecks; and 0 = no visible symptoms.

DNA extraction. For DNA extraction, the fungus was grown in liquid culture (2.4% potato-dextrose agar) for about 3 wk at 20 C. The mycelium was washed three times with ice-cold sterile water, frozen at −80 C for 1 h, and lyophilized for 2 days.

The extraction method was a shortened protocol of the total-DNA minipreparation of Zolan and Pukkila (33) with modifications: the aqueous phase was extracted two times with chloroform/isoamyl alcohol (24:1, v/v). The pellet was rinsed in 1 ml of 70% ethanol after isopropanol precipitation and centrifugation (15 min, 12,000 *g*); after centrifugation the ethanol was discarded, and the pellet was allowed to dry. The pellet was resuspended in 100 µl of Tris-EDTA buffer; RNA digestion was not performed.

Amplification conditions. Amplification reaction volumes were 15 µl, containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ (Stähelin, Basel, Switzerland), 100 µM each of dATP, dCTP, dGTP and dTTP (Boehringer GmbH, Mannheim, Germany), 0.3 µM primer, 5 ng of genomic DNA, and 1 unit of Super*Taq* DNA polymerase (Stähelin). Amplification was performed in a Perkin-Elmer Cetus (Norwalk, CT) Gene Amp PCR system 9600 programmed as described by Koller et al (11). Amplification products were electrophoresed in 1.5% agarose (Bio-Rad Laboratories, Richmond, CA) gels with 0.5× Tris-borate-EDTA buffer and stained with ethidium bromide. The following primers were used for all isolates: E15 (ACGCACAACC), E07 (AGATGCAGCC), U19 (GTCAGTGCGG), and U10 (ACCTCGGCAC) from Operon Technologies Inc., Alameda, CA.

RESULTS

Polymerase chain reaction (PCR). Arbitrary decamer primers can be used to generate polymorphic amplified segments of genomic DNA that can differentiate *V. inaequalis* isolates (26,29). Out of 60 decamer primers, we found four (E15, E07, U10, and U19) that generated enough information (18 polymorphic bands: E15 showed nine, E07 two, U10 four, and U19 three polymorphisms) to differentiate 17 of 21 isolates from 1992 and 32 of 44 isolates from 1993 (from the 60 lesions collected in 1993, 16 monoconidial isolates did not grow in culture). In 1992 four isolates from Spartan (Spa 1, 4, 6, and 7) and in 1993 four isolates from Spartan (Spa 8, 10, 13, and 14), three each from Maigold (Mai 3, 4, and 6) and Boskoop (Bos 8, 9, and 14), and two from Glockenapfel (Glo 5 and 6) could not be distinguished within a particular cultivar set, but it was always possible to distinguish isolates originating from different cultivars. The indistinguishable isolates were likely clonal secondary infections, or they originated from overwintering mycelium or conidia as previously observed (3,14). One Maigold lesion showed two distinguishable spore types (Mai 4 and 5). To distinguish isolates, we selected only polymorphisms represented by strongly and consistently amplified DNA fragments as informational bands (Fig. 1) and by repeating the random amplified polymorphic DNA (RAPD)-PCR at least three times for each isolate; variation in minor bands resulting from inconsistent amplification was excluded.

Cross-infections. The reactions of apple cultivars against conidial mixtures and monoconidial inoculum of *V. inaequalis*

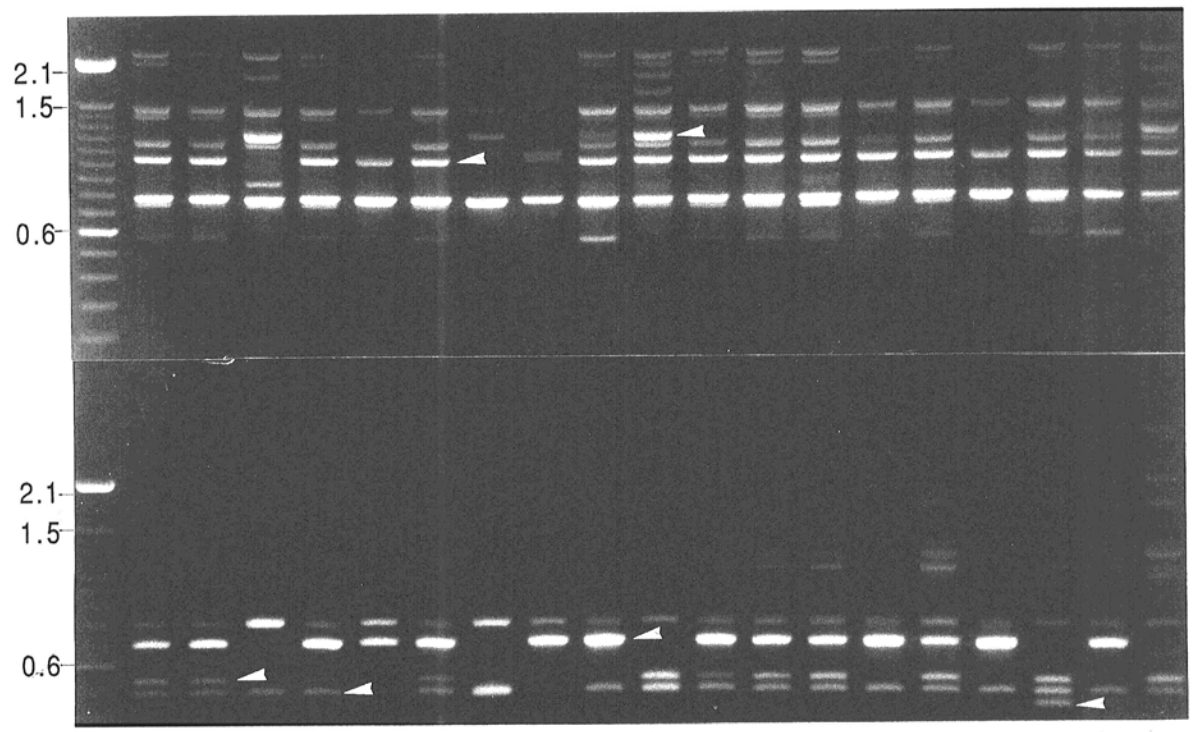

Fig. 1. Amplified DNA segments of the first 19 monoconidial reisolations of *Venturia inaequalis*, after one asexual propagation cycle on apple cultivar Boskoop beginning with a mixture of 10 isolates from Boskoop. The amplification was made with primers E15 (top) and U19 (bottom) in the same polymerase chain reaction. Some polymorphisms used for identification are marked with arrows. The GIBCO Diagnostics (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD) 100-bp ladder is shown on the left side.

isolated from different host cultivars were highly dependent on the particular cultivar. Strongly sporulating lesions were observed when the inoculum originated from the same host cultivar (Tables 1 and 2). On Maigold and Golden Delicious, all inocula showed sporulating lesions (Table 2). The remaining cultivars exhibited differential interactions based on the origin of the inoculum. None of the inocula caused strongly sporulating lesions on Glockenapfel or Boskoop, except when the inoculum originated from these same host cultivars.

Mixed-population trial in 1992. Inoculation with a conidial population consisting of strains originating from three cultivars, Boskoop, James Grieve, and Spartan, followed by three asexual cycles on each cultivar, resulted in a shift in the composition of the population (Fig. 2). The starting frequency of all isolates was expected to be 4.76% (1/21); the four indistinguishable isolates from Spartan (Spa 1, 4, 6, and 7) had a total frequency of 19.05%. From Boskoop, only isolates previously identified as Boskoop isolates were detected; non-Boskoop isolates were not detected. The result was similar on the other two cultivars. After the three asexual propagation cycles, eight isolates were no longer detected (Bos 1 and 6; JG 1, 2, 4, and 7; and Spa 2 and 3). Five isolates were sampled at low frequencies (Bos 4, 5, and 7; JG 3; and Spa 5) on their own cultivars. The five remaining isolates showed an increase in frequency on their respective cultivars; Bos 2 (to 63%) and 3 (to 26%), JG 5 (to 50%) and 6 (to 45%), and the clonal group of Spa 1, 4, 6, and 7 (to 92%). The isolates that occurred at high frequencies were all significantly ($P < 0.05$) different from the expected frequencies (starting frequencies).

Mixed population trial in 1993. DNA fragments of the first 19 reisolations of Boskoop were amplified with primers E15 and U19 (Fig. 1). In this experiment, the relative frequency of the isolates shifted considerably (Fig. 3) from the initial frequency of 10% for all isolates (from the 10 collected lesions). For example, the scab population from and on Idared reduced from 10 to five strains, with one strain dominating (more than 60%; Fig. 3B). The observed distribution of the reisolates among all cultivars was significantly ($P < 0.05$) different from the expected frequency distribution.

TABLE 1. Symptoms caused by *Venturia inaequalis* spores collected during July 1992 from scabbed leaves of three apple cultivars: Boskoop (Bos), James Grieve (JG), and Spartan (Spa)

Origin of inoculum	Symptoms on inoculated host cultivar[a]		
	Bos	JG	Spa
Bos	4	1	2
JG	1	3	2
Spa	0	2	4

[a]Symptoms: 4 = obvious and strong sporulating lesions; 3 = well sporulating but weak chlorotic lesions; 2 = small, chlorotic or weakly sporulating flecks; 1 = small, chlorotic flecks; and 0 = no visible symptoms. Three trees with four inoculation spots were classified. No deviations were observed.

TABLE 2. Symptoms and compatible/incompatible status caused by *Venturia inaequalis* spores collected during May 1993 from scabbed leaves of six apple cultivars: Maigold (Mai), Golden Delicious (Gol), Glockenapfel (Glo), Spartan (Spa), Boskoop (Bos), and Idared (Ida)[a]

Origin of inoculum	Symptoms on inoculated host cultivar[b,c]					
	Mai	Gol	Glo	Spa	Bos	Ida
Mai	4 (+)	4 (+)	1 (−)	3 (+)	1 (−)	4 (+)
Gol	4 (+)	4 (+)	1 (−)	4 (+)	1 (−)	1 (−)
Glo	4 (+)	4 (+)	4 (+)	2 (−)	1 (−)	3 (+)
Spa	3 (+)	4 (+)	1 (−)	3 (+)	1 (−)	4 (+)
Bos	3 (+)	3 (+)	1 (−)	2 (−)	4 (+)	4 (+)
Ida	3 (+)	3 (+)	1 (−)	3 (+)	2 (−)	4 (+)

[a]The inoculum consisted of spores from 10 lesions of each cultivar which were previously propagated as mixtures on their own cultivars.
[b]Symptoms: 4 = obvious and strong sporulating lesions; 3 = well sporulating but weak chlorotic lesions; 2 = small, chlorotic or weakly sporulating flecks; 1 = small, chlorotic flecks; and 0 = no visible symptoms. Three trees with four inoculation spots were classified. No deviations were observed.
[c]Compatibility status: + = compatible, − = incompatible.

DISCUSSION

The purpose of these experiments was not only to investigate differences between the scab populations originating from different apple cultivars but also to detect variability in "parasitic fitness" (2) among strains within a population originating from a particular cultivar at least within the conditions of the experiments. The apple cultivars that were used are all considered susceptible to apple scab in Switzerland. However, the presence and extent of variation for pathogenicity against *M. domestica* within the local pathogen populations has not been characterized. To approach this problem, we used mixed-inoculation experiments with naturally occurring isolates genetically tagged by RAPD markers. This provided a means of clearly determining if there were any large changes in the frequencies of isolates over a few generations. We could only detect large changes, because the final sample sizes were limited to 50 individuals per trial. As a comparison, well-sporulating lesions produce up to 30,000 conidia (24).

Notwithstanding the small sample sizes, the experiments indicated that the interaction between isolates of the apple scab pathogen and apple cultivars is not selectively neutral (Tables 1 and 2; Figs. 2 and 3). There were not only considerable differences in the ability of isolates from a specific cultivar to infect other cultivars (Fig. 2), but also in their relative reproductive ability on the source cultivar (Fig. 3).

The 1992 experiment on cultivars Boskoop, James Grieve, and Spartan revealed cultivar-specific interactions: Isolates were recovered only from the cultivars from which they originated, a result similar to the 1993 cross-infections. Thus, specific pathogen genotypes showed specialization toward particular host genotypes.

These observations are consistent with the gene-for-gene theory (7,25), assuming the presence of specific resistance genes in particular domestic cultivars and corresponding specific virulence genes in the pathogen. The 1993 experiment indicated that when mixtures of isolates are cycled for one asexual generation on the cultivar from which they were originally collected, some iso-

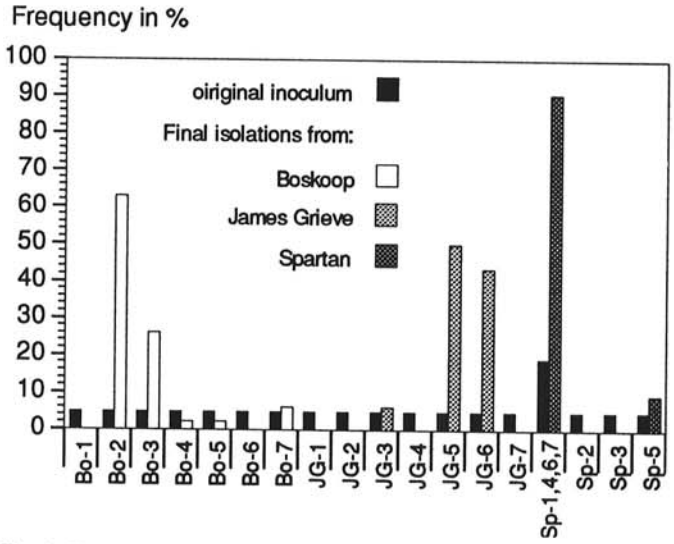

Fig. 2. Frequency of single-spore isolates of *Venturia inaequalis* after three asexual cycles on apple cultivars Boskoop, James Grieve, and Spartan starting from a mixed inoculum of equal amounts of conidia from 21 isolates (seven from each cultivar). The isolates were identified with random amplified polymorphic DNA-polymerase chain reaction. Isolates Spartan 1, 4, 6, and 7 were undistinqushable; Bo 1–7: isolates originating from Boskoop (frequency out of 46); JG 1–7: isolates originating from James Grieve (frequency out of 46); Sp 1–7: isolates originating from Spartan (frequency out of 32).

lates were no longer detected, and others increased dramatically in frequency. The observation that even isolates originating from the same cultivar show differences in fitness is possibly due to the predominant sexual cycle of *V. inaequalis* each winter (5), from which recombination generates new combinations of genes each spring providing considerable genotypic variation. We are continuing to investigate the nature of this variation with genetically controlled crosses among pathogen isolates.

Agricultural implications. Previous studies (15,17–21,27) showed that different individuals of the scab fungus possess different abilities to attack various cultivars, and thus, it is expected that different cultivars impose different selection pressures on a variable *V. inaequalis* population. Newly introduced "susceptible" apple cultivars are often more resistant than those which are common in a particular region. Golden Delicious seems to be resistant in some regions of Italy (10), where it is infrequent, whereas it is the most susceptible cultivar in Switzerland (9). A resistant cultivar can exert strong selection (12), so that with an increase in the number of trees of the cultivar and the period for which it is grown in a region, virulent strains of the pathogen are likely to increase in frequency until the cultivar is susceptible to the overwhelming proportion of the local fungal population.

Our results are consistent with this reasoning, showing that the most frequent apple cultivar in Switzerland, Golden Delicious,

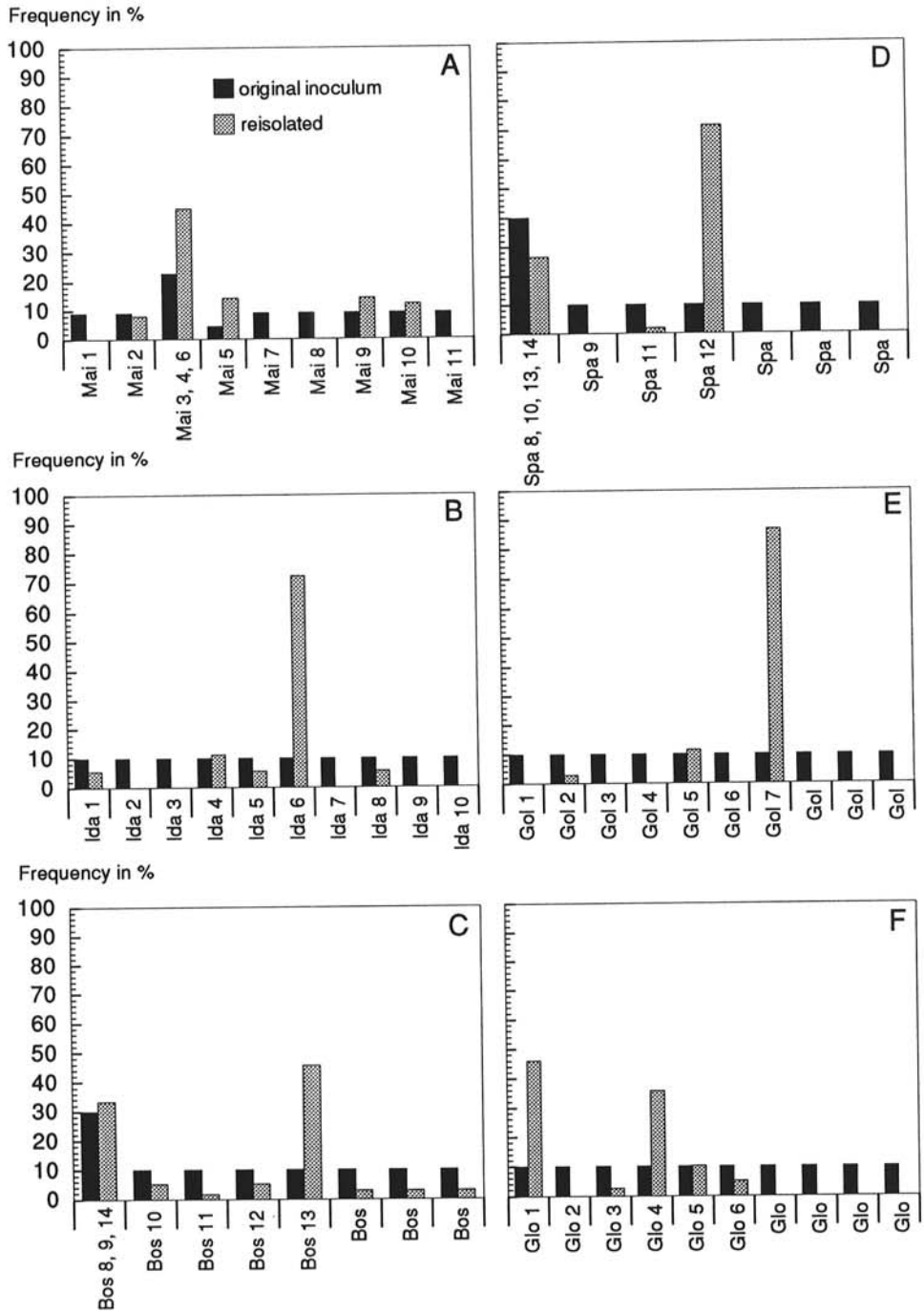

Fig. 3. Frequency of single-spore isolates of *Venturia inaequalis* after one asexual cycle of a mixture of equal amounts of conidia from 10 lesions from apple cultivars: **A,** Mai, isolates originating from Maigold (frequency out of 49); **B,** Ida, isolates originating from Idared (frequency out of 36); **C,** Bos, isolates originating from Boskoop (frequency out of 57); **D,** Spa, isolates originating from Spartan (frequency out of 49); **E,** Gol, isolates originating from Golden Delicious (frequency out of 36); and **F,** Glo, isolates originating from Glockenapfel (frequency out of 39). The isolates were identified with random amplified polymorphic DNA-polymerase chain reaction. The isolates represented by one column showed no differences. The isolates without a number were those that disappeared. One lesion of Maigold revealed two spore types during identification: Mai 4 and 5.

and its near relative, Maigold, are attacked severely by all the isolates we tested (Table 2). The other cultivars exhibited resistance to some isolates but less resistance to the isolates that originated from them, consistent with cultivar-specific adaptation. The considerable variation in pathogen response to different cultivars probably arises because of limited movement of the pathogen among orchards, allowing local adaptation to build up in relative isolation.

Can this variation be exploited to improve disease control or breeding strategies? One possibility is a system based on the concept of mixtures of cultivars with functionally different resistances (30). As far as we know, no major experiments have been made yet with apple, but computer simulation models show a reduction of about 70% of the lesions after six asexual generations in "mixed" compared with "pure" orchards (4,8). In cereals, planting strategies based on mixing host resistances have been proposed and have proven valuable (30,31). In apples, where mixed plantings of cultivars can be economically tolerated, there may be considerable benefit realized by confronting the pathogen population with different host genotypes simultaneously and, thereby, slowing down the process of adaptation and the establishment of highly aggressive strains in comparison with a monocultivar system.

Another possibility for using differential resistance of susceptible apple cultivars could be to introduce one or more of the genes responsible for resistances into a single cultivar (pyramiding of genes) by conventional breeding methods or by genetic engineering. The disadvantage here is that the genetic diversity of different apple cultivars (and so diversity in the apple population used for commercial production) is reduced, and the desired characters in different host clones responsible for slow adaptation of the pathogen population are lost. Such characters could include the presence of phenolic compounds and differences in cell wall structure. Moreover, the rate of the evolution of virulence of the *V. inaequalis* population cannot be predicted, because no further studies of the inheritance were made in terms of ability to accumulate several virulence genes into one fungal strain. However, following other host-pathogen systems, it is likely that complex strains capable of overcoming the pyramidal resistances may soon appear, because the individual virulences are already available in the population and sexual recombination is common (12,31,32).

To improve disease control by exploiting variation in pathogenicity and resistance, further studies will be made in and among orchards planted with susceptible apple cultivars for estimation of the variability of virulence in *V. inaequalis* populations. New strategies for the management of apple depend on the behavior of the virulence characters of the fungus during the sexual cycle. For this reason, inheritance studies of isolates with different virulences will be made with the aim of characterizing the virulence and parasitic fitness of *V. inaequalis*. The breeding strategies should be based on the possibility of recognizing the resistances maintained in susceptible cultivars by serial testing of progenies with appropriate single or mixed pathotypes of *V. inaequalis* or by marker-assisted screening (13).

Agricultural production should be oriented toward environmental safety and sustainability, which means low inputs and maintenance of biological diversity. The discovery of resistances in mainly old and "susceptible" apple cultivars could help to achieve this aim.

LITERATURE CITED

1. Aderhold, R. 1899. Auf welche Weise können wir dem immer weiteren Umsichgreifen des Fusicladiums in unseren Apfelkulturen begegnen und welche Sorten haben sich bisher dem Pilz gegenüber am widerstandsfähigsten gezeigt. Pomol. Monatshefte 45:266-272.
2. Andrivon, D. 1993. Nomenclature for pathogenicity and virulence: The need for precision. Phytopathology 83:889-890.
3. Becker, C. M., Burr, T. J., and Smith, C. A. 1992. Overwintering of conidia of *Venturia inaequalis* in apple buds in New York orchards. Plant Dis. 76:121-126.
4. Blaise, Ph., and Gessler, C. Cultivar mixtures in apple orchards as a mean to control apple scab? Norw. J. Agric. Sci. In press.
5. Boone, D. M. 1971. Genetics of *Venturia inaequalis*. Annu. Rev. Phytopathol. 9:297-318.
6. Crosby, J. A., Janik, J., Pecknold, P. C., Korban, S. S., O'Connor, P. A., Ries, S. M., Goffreda, J., and Voordeckers, A. 1992. Breeding apples for scab resistance: 1945–1990. Fruit Var. J. 46:145-166.
7. Flor, H. H. 1956. The complementary genic systems in flax and flax rust. Pages 29-54 in: Advances in Genetics, vol. 8. M. Demerec, ed. Academic Press, New York.
8. Gessler, C. Biology and biotechnology in strategies to control apple scab. Norw. J. Agric. Sci. In press.
9. Götz, G., and Silbereisen, R. 1989. Obstsorten-Atlas. Eugen Ulmer Verlag, Stutgart, Germany.
10. Govi, G. 1966. Sensibilità varietale del melo alle infezioni di *Venturia inaequalis*. Phytopathol. Mediterr. 55:145-146.
11. Koller, B., Lehmann, A., McDermott, J. M., and Gessler, C. 1993. Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. 85:901-904.
12. Leonard, K. J. 1987. The host population as a selective factor. Pages 163-179 in: Populations of Plant Pathogens: Their Dynamics and Genetics. M. S. Wolfe and C. E. Caten, eds. Blackwell Scientific Publications, Oxford.
13. Melchinger, A. E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed. 104:1-19.
14. Moosherr, W. 1990. Untersuchungen zu superfizillem Schorf und Knospenschorf (*Venturia inaequalis* (Cke.) Wint.) beim Apfel. Ph.D. thesis. University of Hohenheim, Germany.
15. Palmiter, D. H. 1933. Variability in monoconidial cultures of *Venturia inaequalis*. Phytopathology 24:22-47.
16. Parisi, L., Lespinasse, Y., Guillaumes, J., and Kruger, J. 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the Vf gene. Phytopathology 83:533-537.
17. Rudloff, C. F. 1935. *Venturia inaequalis* (Cooke) Aderhold, III. Zur Formenmannigfaltigkeit des Pilzes. Gartenbauwissenschaft 9:105-120.
18. Schmidt, M. 1935. *Venturia inaequalis* (Cooke) Aderhold. IV. Weitere Beiträge zur Rassenfrage beim Erreger des Apfelschorfes. Gartenbauwissenschaft 9:364-389.
19. Schmidt, M. 1936. *Venturia inaequalis* (Cooke) Aderhold. V. Weitere Untersuchungen über die auf verschiedenen Bäumen lebenden Populationen des Apfelschorfes. Gartenbauwissenschaft 10:422-427.
20. Schmidt, M. 1936. *Venturia inaequalis* (Cooke) Aderhold. VI. Zur Frage nach dem Vorkommen physiologisch spezialisierter Rassen beim Erreger des Apfelschorfes. Erste Mitteilung. Gartenbauwissenschaft 10:478-499.
21. Schmidt, M. 1940. *Venturia inaequalis* (Cooke) Aderhold. X. Zur Vererbung der morphologischen Merkmale auf künstlichem Substrat und der Aggressivität gegenüber bestimmten Wirten bei Einsporenherkünften des Apfelschorfpilzes. Gartenbauwissenschaft 15:118-130.
22. Shay, J. R., and Williams, E. B. 1956. Identification of three physiological races of *Venturia inaequalis*. Phytopathology 46:190-193.
23. Sierotzki, H., Eggenschwiler, M., McDermott, J., and Gessler, C. Specific virulence of isolates of *Venturia inaequalis* on "susceptible" apple cultivars. Norw. J. Agric. Sci. In press.
24. Smith, C. A., and MacHardy, W. E. 1993. The production of conidia by *Venturia inaequalis* on 7 apple cultivars. (Abstr.) Phytopathology 83:247.
25. Vanderplank, J. E. 1982. Host-pathogen interactions in plant disease. Academic Press, New York.
26. Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrarily primers. Nucleic Acid Res. 18:7213-7218.
27. Wiesmann, R. 1931. Untersuchungen über Apfel und Birnschorfpilz *Fusicladium dendriticum* (Wallr.) Fckl. und *Fusicladium pirinum* (Lib.) Fckl. sowie die Schorfanfälligkeit einzelner Apfel und Birnsorten. Landwirtsch. Jahrb. Schweiz 45:109-164.
28. Williams, E. B., and Brown, A. G. 1968. A new physiological race of *Venturia inaequalis*, incitant of apple scab. Plant Dis. Rep. 52:799-801.
29. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.
30. Wolfe, M. S. 1985. The current status and prospects of multiline cultivars and cultivar mixtures for disease resistance. Annu. Rev. Phytopathol. 23:251-273.
31. Wolfe, M. S. 1992. Barley diseases: Maintaining the value of our varieties. 6th Int. Barley Genet. Symp. 2:1055-1067.
32. Wolfe, M. S., and Gessler, C. 1992. The use of resistances genes in breeding: Epidemiological considerations. Pages 3-23 in: Genes involved in Plant Defense. T. Boller and F. Meins, eds. Springer Verlag, Vienna.
33. Zolan, M. E., and Pukkila, P. J. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Mol. Cell. Biol. 6:195-200.