Stanley Freeman, Talma Katan, and Ezra Shabi
The Volcani Center, Bet Dagan, Israel

Characterization of *Colletotrichum* Species Responsible for Anthracnose Diseases of Various Fruits

Filamentous fungi of the genus *Colletotrichum* and its teleomorph *Glomerella* are considered major plant pathogens worldwide. They cause significant economic damage to crops in tropical, subtropical, and temperate regions. Cereals, legumes, ornamentals, vegetables, and fruit trees may be seriously affected by the pathogen (3). Although many cultivated fruit crops are infected by *Colletotrichum* species, the most significant economic losses are incurred when the fruiting stage is attacked. *Colletotrichum* species cause typical disease symptoms known as anthracnose, characterized by sunken necrotic tissue where orange conidial masses are produced. Anthracnose diseases appear in both developing and mature plant tissues (4). Two distinct types of diseases occur: those affecting developing fruit in the field (preharvest) and those damaging mature fruit during storage (postharvest). The ability to cause latent or quiescent infections has grouped *Colletotrichum* among the most important postharvest pathogens. Species of the pathogen appear predominantly on aboveground plant tissues; however, belowground organs, such as roots and tubers, may also be affected.

In this article, we deal in particular with methods used to identify and characterize *Colletotrichum* species and genotypes from almond, avocado, and strawberry, as examples, using traditional and molecular tools. The three pathosystems chosen represent different disease patterns of fruit-associated *Colletotrichum.*

Corresponding author: Stanley Freeman, Department of Plant Pathology, ARO, The Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel; TEL: 972-3-9683537, FAX: 972-3-9683543, E-mail: freeman@netvision.net.il

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Multiple Species on a Single Host

Numerous cases have been reported in which several *Colletotrichum* species or biotypes are associated with a single host. For example, avocado and mango anthracnose, caused by both *C. acutatum* and *C. gloeosporioides*, affect fruit predominantly as postharvest diseases (25,40,41). Strawberry may be infected by three *Colletotrichum* species, *C. fragariae*, *C. acutatum*, and *C. gloeosporioides*, causing anthracnose of fruit and other plant parts (31). Almond and other deciduous fruits may be infected by *C. acutatum* or *C. gloeosporioides* (Table 1) (1,5,46,50). Citrus can be affected by four different *Colletotrichum* diseases (61): postbloom fruit drop and key lime anthracnose, both caused by *C. acutatum*, and shoot dieback and leaf spot, and postharvest fruit decay, both caused by *C. gloeosporioides*. Additional examples of hosts affected by multiple *Colletotrichum* species include coffee, cucurbits, pepper, and tomato.

Single Species on Multiple Hosts

It is common to find that a single botanical species of *Colletotrichum* infects multiple hosts. For example, *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (teleomorph: *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk), which is considered a cumulative species and forms the sexual stage in some instances, is found on a wide variety of fruits, including almond, avocado, apple, and strawberry (Table 2) (6,15,31,46). Likewise, *C. acutatum* J.H. Simmonds has been reported to infect a large number of fruit crops, including avocado, strawberry, almond, apple, and peach (1,5,16,25,27). Examples of other species with multiple host ranges include *C. coccodes*, *C. capsici*, and *C. dematium* (14,56).

Importance of Species Identification and Characterization

Diagnostics/etiology. Due to the complicated situations just described, a *Colle-*

Table 1. Comparison between two populations of *Colletotrichum* isolated from almond fruit in Israel and California

Character	Israel	California
Species	*C. gloeosporioides*	*C. acutatum*
Morphology in culture	White to gray	Gray to pink
Sexual stage	Absent	Absent
Optimal growth temperature	20 to 22°C	24 to 26°C
Average growth rate (and range) on potato dextrose agar at optimal temperature (mm/day)	2.2 (1.6 to 2.8)	7.7 (4.9 to 9.9)
Infected plant part	Immature fruit	Immature fruit
Vegetative compatibility grouping [a]	Single	Single
Number of molecular genotypes [b]	Single	Single
Benomyl sensitivity	Insensitive [c]	Insensitive [d]

[a] Isolates from California were not vegetatively compatible with isolates from Israel.
[b] Isolates from California were distinct from Israeli isolates based on arbitrarily primed polymerase chain reaction (ap-PCR).
[c] Benomyl (5 µg/ml) sensitivity based on fungicide-amended media (Fig. 2).
[d] Benomyl (1,200 µg/ml) sensitivity based on fungicide disk assays (1).

totrichum species–host combination involved in a given anthracnose incidence alone is often insufficient as a diagnostic indicator of disease etiology. Consequently, to resolve ambiguous disease cases, studies have focused first on taxonomic identification of *Colletotrichum* species, and second on characterization of subpopulations within each species. Differentiating between *Colletotrichum* species responsible for disease epidemics is vital for developing and implementing effective control strategies. A precise determination of the etiology of different *Colletotrichum* diseases is essential for understanding the epidemiology of these diseases. It is of paramount importance to investigate whether the same or different pathogens are associated with diseases on different tissues. For example, citrus infection by *C. acutatum* will cause postbloom drop; whereas *C. gloeosporioides* is predominantly a postharvest pathogen of the same citrus species that may survive in the field as a common saprophyte (61). For effective control, it is important to validate that a specific species is indeed the causal agent of a disease that may occur in different geographic locations worldwide. Likewise, breeding for resistance to a given species (22,29,55) depends on accurate etiology of the pathogen in question.

Host specificity. Differentiation between *Colletotrichum* species based on host range or host of origin may not be a reliable criterion for fungi of this genus, since taxa such as *C. gloeosporioides, C. dematium, C. acutatum, C. graminicola,* and others infect a broad range of host plants. Some taxa appear to be restricted to host families, genera or species within those families, or even cultivars (e.g., bean [51]); whereas others have more extensive host ranges. On the other hand, certain highly specific strains of *Colletotrichum* have been successfully developed as commercial mycoherbicides (e.g., *C. gloeosporioides* f. sp. *aeschynomene* for the control of northern jointvetch, a weed in rice and soybean [57]).

Table 2. Comparison between isolates of *Colletotrichum gloeosporioides* from almond and avocado

Character	Almond[a]	Avocado[b]
Morphology in culture	White to gray	White, gray to black
Sexual stage	Absent	Present
Optimal growth temperature	20 to 22°C	26 to 28°C
Average growth rate (and range) on potato dextrose agar at optimal temperature (mm/day)	2.2 (1.6 to 2.8)	6.4 (3.8 to 12.0)
Infected plant part	Immature fruit (dry rot)	Leaves, twigs, immature and mature fruit (soft rot)
Latent infections	Absent	Present
Vegetative compatibility grouping	Single	Multiple
Number of molecular genotypes	Single	Multiple
Benomyl sensitivity	Insensitive	Sensitive

[a] From Israel.
[b] From Israel and the United States.

Fig. 1. Typical anthracnose symptoms caused by *Colletotrichum gloeosporioides* (A, B, and C) and *C. acutatum* (D): (A) on immature almond fruit, (B) wilting of distal leaves associated with almond fruit infection, (C) on mature avocado fruit (courtesy D. Prusky, Volcani Center, Israel), and (D) on unripe and ripe strawberry fruit. Arrows indicate sporulating lesions.

Traditional and molecular identification of *Colletotrichum.* Traditional methods for identifying species of *Colletotrichum* have relied primarily on morphological differences such as colony color, size and shape of conidia, optimal temperature, growth rate, presence or absence of setae, and existence of the teleomorph, *Glomerella* (24,52,56,59). However, due to environmental influences on the stability of morphological traits and the existence of intermediate forms, these criteria are not always adequate for reliable differentiation among *Colletotrichum* species.

Vegetative compatibility, a mechanism that controls genetic isolation of populations, has been used extensively to examine genetic relatedness in a number of plant pathogenic fungi (34). Vegetative compatibility grouping (VCG) has been used for subspecies analysis of *Colletotrichum* species, including *C. dematium* from spinach (11), *C. orbiculare* from cucurbits (60), *C. gloeosporioides* from almond (33), *C. graminicola* from maize (58), and *C. acutatum* from strawberry (16).

Over the past decade, various molecular techniques have been used successfully to complement the above-mentioned approaches for reliable discrimination among species. For example, arbitrarily primed polymerase chain reaction (ap-PCR) and polymorphisms in nuclear DNA, ribosomal DNA (rDNA), mitochondrial DNA (mtDNA), and A+T-rich DNA have been utilized to differentiate among populations of *C. acutatum, C. gloeosporioides, C. coccodes, C. fragariae, C. kahawae, C. magna, C. orbiculare,* and other species (9,12,19,28,32,49,54). Likewise, the application of CHEF gel analysis and molecular markers to Australian isolates of *C. gloeosporioides* on the tropical pasture legume, *Stylosanthes* spp., indicated that two distinct clonal populations appear to exist (26,37,38). In addition, the genetic complexity of *Colletotrichum* strains infecting various temperate and tropical fruits has been shown using these techniques (5,17,19,39).

Anthracnose of Almond, Avocado, and Strawberry

In this and the next sections, we will focus on three pathosystems of anthracnose diseases, occurring on fruit of almond, avocado, and strawberry (Fig. 1), which represent different disease patterns of *Colletotrichum.* Species of the pathogen on almond, a deciduous crop, infect at initial fruiting stages but do not appear to attack mature fruit or other plant organs (Fig. 1A and B) (1,7,13,46). In contrast, avocado is an evergreen crop, in which the anthracnose pathogens cause postharvest decay due to latent or quiescent infections initiating in the field (Fig. 1C) (6,41,42). Unlike almond and avocado, which are perennial, strawberry is predominantly an annual crop. Strawberry anthracnose, caused by either *C. gloeosporioides, C. acutatum,* and/or *C. fragariae,* is responsible for serious damage on foliar and fruiting plant parts, as well as for root necrosis (16,31,36,52). Damage to strawberry crops is not limited to a certain growth period or storage conditions, and anthracnose may appear throughout the season (Fig. 1D).

Although almond and avocado crops are cultivated in close proximity in Israel, where anthracnose of both crops, caused by *C. gloeosporioides,* occurs during the spring season, it was not clear whether cross-infection resulted in the field. Likewise, strawberry, infected by *C. acutatum,* is cultivated in the same geographic regions and under the same climatic conditions as those of avocado and almond crops in Israel. Furthermore, a recent report has characterized *C. acutatum* as the pathogen responsible for almond anthracnose in California in areas where strawberry and peach anthracnose occur (1). The question raised therefore is whether the pathogens responsible for anthracnose in these locations are host-specific, or whether the same pathogen is the causal agent of disease occurring in the three crops. The methods employed in answering this question may be applied to similar situations where single *Colletotrichum* species attack multiple hosts or where multiple *Colletotrichum* species appear on a single host, as described.

The approaches used to identify and characterize the anthracnose pathogens in Israel included (i) traditional methods based on various morphological criteria as well as benomyl sensitivity, (ii) cross-inoculation of different isolates on various fruits to determine host specificity, (iii) VCG testing to determine relatedness of isolates within and between species, and (iv) molecular analyses to identify *Colletotrichum* species and to characterize subpopulations within species.

Identification of Species and Characterization of Subspecies

Characterization of *Colletotrichum* species has relied on a number of criteria, including morphology, optimal growth temperature, vegetative compatibility, benomyl sensitivity, and molecular methods. In this section, we elaborate on the approaches used to differentiate between isolates of *Colletotrichum* responsible for anthracnose on almond, avocado, and strawberry crops in Israel.

Traditional methods. In general, conidia of *C. acutatum* are elliptic-fusiform in shape; whereas conidia of *C. gloeosporioides* are oblong with obtuse ends. However, this criterion, as well as the size of conidia, is not consistent enough for discerning species.

Numerous reports suggest that isolates of *C. acutatum* grew at a significantly slower rate than isolates of *C. gloeosporioides* (5,50,52). However, growth rate of *C. gloeosporioides* isolates from almond in Israel was slow and did not differ significantly from that of *C. acutatum* from strawberry. In fact, growth rate and optimal temperature have been useful for differentiating between subpopulations of *C. gloeosporioides* from avocado and almond crops (Table 2) (47).

Colony shape and color of cultures may vary considerably within and between species. For instance, most isolates of *C. acutatum* (including the almond pathogen from California) show a red-salmon pigmentation in reverse culture; whereas the coloration of *C. gloeosporioides* cultures varies from white to dark gray (Table 1). Several morphological types have been observed within a population of *C. gloeosporioides* from avocado grown in

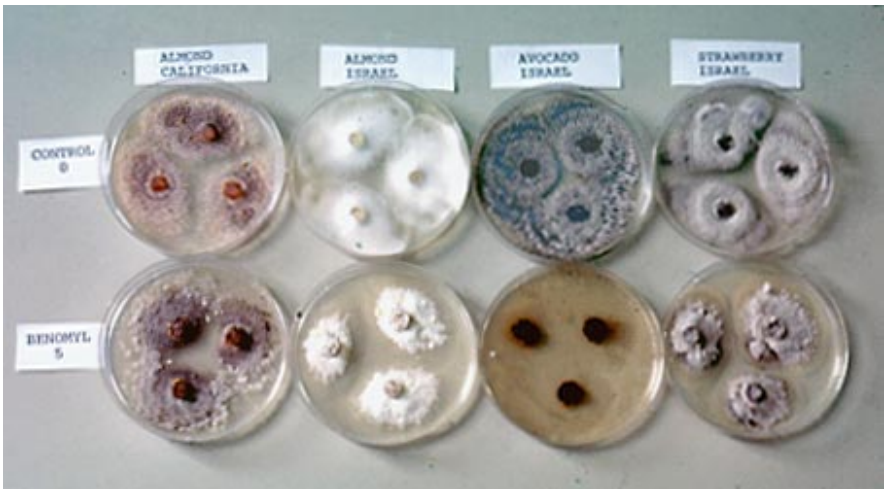

Fig. 2. Benomyl (5 µg/ml) sensitivity assay of *Colletotrichum acutatum* isolates from almond (California) and strawberry, and *C. gloeosporioides* isolates from almond (Israel) and avocado. Only the avocado isolate is sensitive to benomyl.

culture (T. Katan, *unpublished*): (i) conidial type, with masses of conidia and no aerial mycelium; (ii) flat mycelial type, with very little aerial mycelium or conidia; (iii) mycelial type, with dense aerial mycelium; (iv) conidial-mycelial type, with dense aerial mycelium and masses of conidia; (v) protoperithecial type, similar to type iv but also producing dark protoperithecia-like hyphal aggregates; and (vi) perithecial type, with simple or complex perithecia. The existence of the teleomorph *Glomerella* may sometimes help in differentiating between species; however, the sexual stage has only been observed in some isolates of *C. gloeosporioides* from avocado (T. Katan and S. Freeman, *unpublished*), but not from almond. In contrast to the morphological heterogeneity observed among the *C. gloeosporioides* isolates from avocado, the population originating from almond in Israel had uniformly growing white-to-gray colonies. It may be concluded that colony color and shape of conidia are unsatisfactory methods for identification of species, primarily due to the diversity of these characteristics in *C. gloeosporioides*, which is indicative of its heterogeneity.

Benomyl sensitivity. Screening of fungicide sensitivity has been used primarily to estimate the potential of such compounds for chemical control (18,30). It was found that in some cases, fungicide sensitivity can also be used for species and subspecific grouping in *Colletotrichum.* In general, *C. gloeosporioides* isolates are considered highly sensitive to benomyl; whereas *C. acutatum* isolates are relatively insensitive (5,9,35). According to our studies, *C. gloeosporioides* from avocado and almond differ in their sensitivity to benzimidazole fungicides: almond isolates are insensitive to benzimidazoles, retaining 40 to 50% growth at 0.5 µg/ml (benomyl), with little further reduction at 50 µg/ml, compared with complete growth inhibition of isolates from avocado at 5 µg/ml (Fig. 2) (45–47). Likewise, *C. acutatum* isolates from strawberry grown in Israel and California, and from almond grown in California, were insensitive to the above-mentioned concentrations of benomyl (Fig. 2). Additional studies have shown that *C. acutatum* from almond, peach, and strawberry crops from California is insensitive to benomyl (1). This simple method can therefore be utilized for additional characterization of local populations of *Colletotrichum* associated with particular anthracnose diseases (1,9,35).

Host Specificity

Cross-infection potential has been reported among different species of *Colletotrichum* and genotypes of *C. gloeosporioides* on a variety of tropical, subtropical, and temperate fruits under artificial inoculation conditions. For example, isolates of

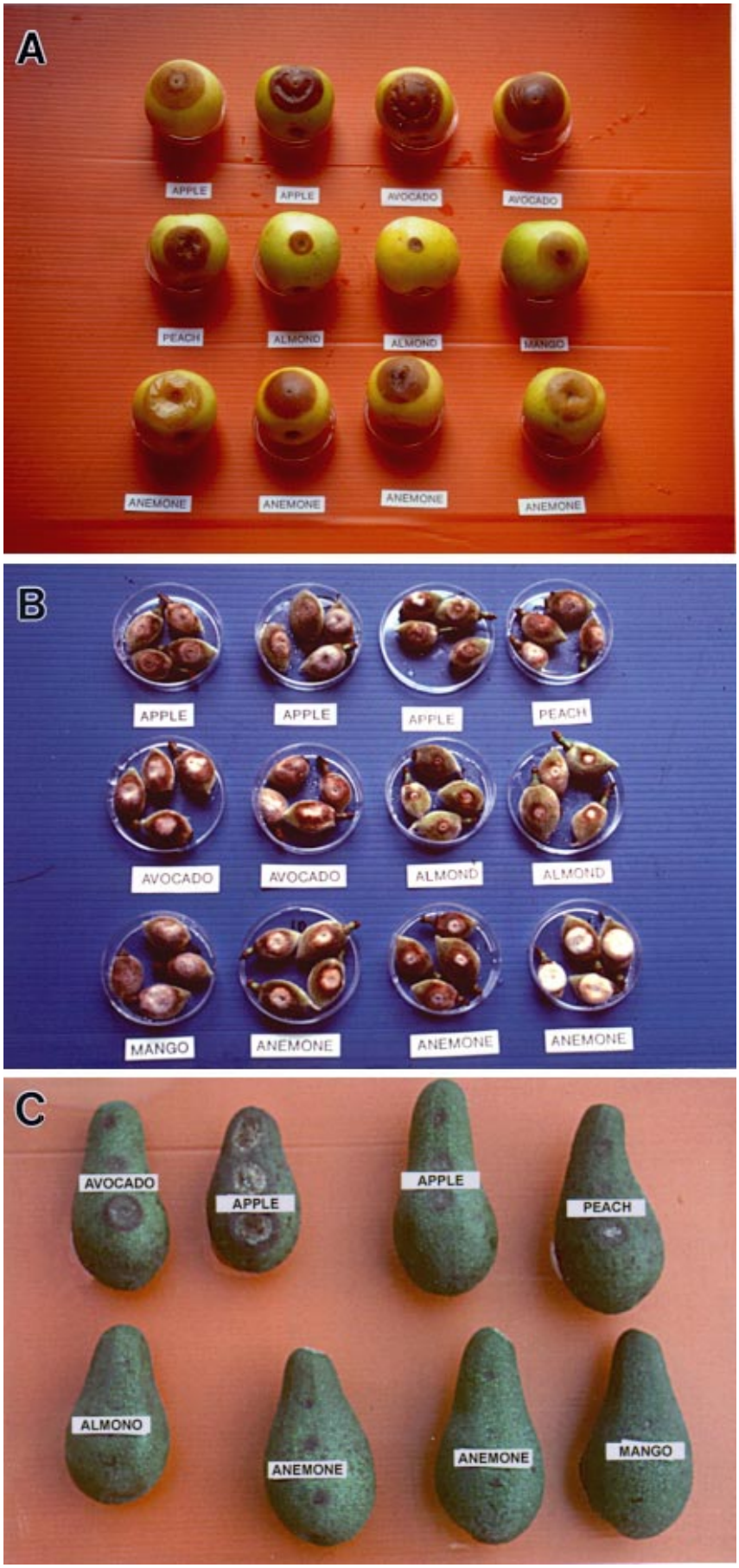

Fig. 3. Artificial inoculation of (A) apple (cv. Golden Delicious), (B) almond (cv. Neplusultra), and (C) avocado (cv. Fuerte) fruits with *Colletotrichum* isolates from various hosts (as indicated by attached labels).

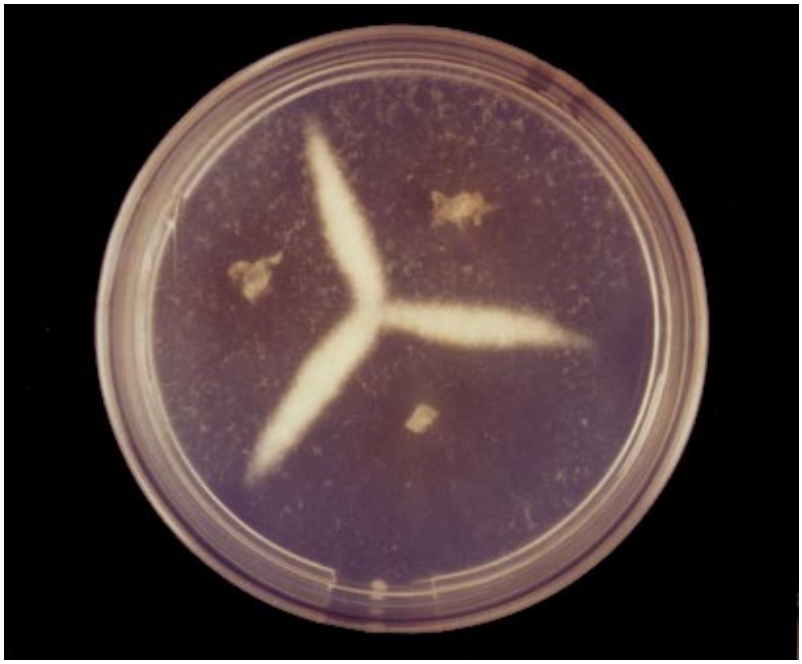

Fig. 4. Formation of prototrophic heterokaryons between complementary *nit* mutants of three isolates of *Colletotrichum gloeosporioides* from almond (Israel) belonging to a single vegetative compatibility group.

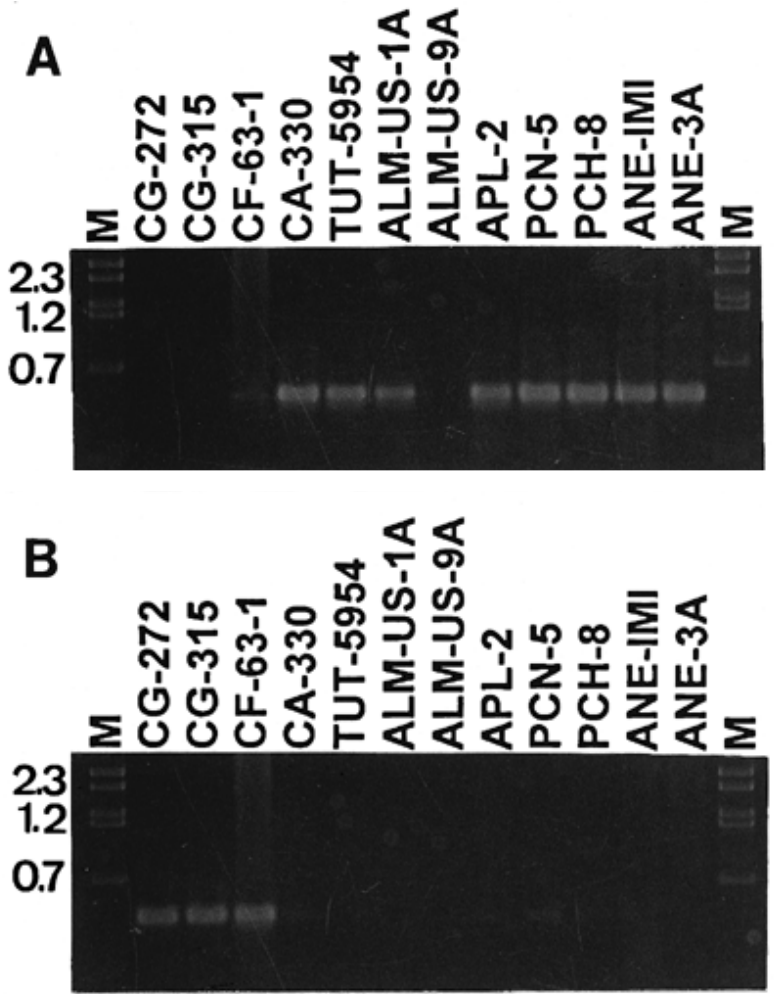

Fig. 5. Amplification of specific DNA fragments from isolates of *Colletotrichum* as specified in Figure 8. Primer pairs CaInt2 and ITS4 are specific for *C. acutatum* (A), and CgInt and ITS4 are specific for *C. gloeosporioides* (B). Lanes M contain DNA size markers in kilobases.

C. acutatum and *C. gloeosporioides* from a variety of temperate fruits caused disease symptoms that were visually indistinguishable when inoculated on detached peach fruits (5). *C. gloeosporioides* isolates from seven tropical fruit crops were also shown to cross-infect alternate detached leaves and fruits, and the success of infection appeared to be dependent on inoculum density (2). In studies conducted in Israel, it was of interest to determine whether avocado anthracnose was caused by the same subspecies of *C. gloeosporioides* as that responsible for almond anthracnose, since almond groves are situated alongside avocado plantations in some cultivation areas. Similarly, other crops, such as anemone, apple, mango, and strawberry, are known to be susceptible to *Colletotrichum* in Israel. To determine the potential of cross-infection, isolates from different crops were cross-inoculated on various hosts.

Our study showed that *C. gloeosporioides* isolates from almond, apple, avocado, and mango, as well as *C. acutatum* isolates from anemone, apple, and peach, infected detached fruits of the other hosts, including apple (two varieties), avocado, almond, mango, and nectarine (Fig. 3) (21). These results demonstrated cross-infection potential between two species, *C. gloeosporioides* (including representatives of distinct subpopulations from almond, apple, avocado, and mango) and *C. acutatum* (from apple and peach), on a variety of fruit species. However, these experiments were conducted under the extreme conditions commonly applied in artificial inoculations; whereas isolations from naturally infected fruit provided no evidence that cross-infection had occurred under field conditions.

VCG. Studies of vegetative compatibility offer another approach to determining genetic relatedness in anamorphic populations of *Colletotrichum* species (8,11,60). Since the exchange of genetic material requires hyphal anastomosis, vegetatively compatible isolates are expected to be more similar to one another, thereby constituting a distinct genetic population (34,43).

VCG studies were conducted with isolates of *Colletotrichum* from almond (from Israel and the United States), avocado (Israel), and strawberry (Israel) crops, to determine the genetic relatedness within and between populations. These Israeli isolates of *C. gloeosporioides* and Californian isolates of *C. acutatum* from almond belonged to single, distinct VCGs and formed complementary heterokaryons between mutants from different isolates only within each population (Fig. 4) (45). Almond isolates from Israel were not compatible with local isolates of *Colletotrichum* from avocado, indicating that the almond population constitutes a distinct subspecific group within *C. gloeosporioi-*

des (33). On the other hand, *C. gloeosporioides* from avocado comprises multiple VCGs, and coupled with its diverse morphology, further indicates heterogeneity of this population (S. Freeman and T. Katan, *unpublished*). In contrast, isolates of *C. acutatum* from different plant parts of strawberry, including fruit and roots, originating from different regions were all vegetatively compatible with one another, indicating uniformity and clonality of the local pathogen population (16). Although the VCG method cannot be used for the taxonomic classification of *Colletotrichum* species, it is useful for delineating asexually reproducing subpopulations within species.

Molecular techniques. Since traditional methods have not been satisfactory for differentiating between species and subspecies of *Colletotrichum*, molecular approaches have gained popularity over the last decade. Molecular methods have been employed successfully to differentiate between populations of *Colletotrichum* from many hosts in general, and in our case, from almond, avocado, and strawberry in particular.

A. Ribosomal DNA (rDNA) analysis. rDNA genes appear as multiple copies in the genome. Due to lesser conservation of sequence, the nontranscribed and internal transcribed spacer (ITS) regions between the small and large nuclear rDNA subunits are suitable for detection of recent evolutionary divergence within *Colletotrichum.* In our work, limited restriction digest analyses of PCR-amplified rDNA were used to differentiate representative isolates of *C. gloeosporioides* from a diverse host range, from rDNA of *C. acutatum* from strawberry (17).

Species-specific primers have been designed primarily according to dissimilarities in the sequence of the ITS regions of representative isolates of *Colletotrichum* from different species (9,28,32,39,53). We have used such primers to differentiate between *C. acutatum* and *C. gloeosporioides* (Fig. 5). As can be seen in Figure 5A, a single PCR-amplified fragment of 490 bp was evident in isolates of *C. acutatum* from a broad host range, but not in *C. gloeosporioides* isolates. Similarly, using primers specific for *C. gloeosporioides* and *C. fragariae,* isolates of these two species were similar to one another but could be distinguished from those of *C. acutatum* by amplification of a specific 450-bp fragment (Fig. 5B). This method also made it possible to distinguish between *C. acutatum* from almond, apple, peach, and strawberry on the one hand and *C. gloeosporioides* from papaya and citrus on the other (1). These results demonstrate that rDNA analysis is adequate as a taxonomic tool for species identification and for diagnostic pathogen detection in planta, but not for revealing subpopulations within a species.

B. Polymorphisms in nuclear DNA. Repetitive DNA elements have been useful for restriction fragment length polymorphism (RFLP) grouping of various isolates of *Colletotrichum* species (37,44). For example, a repetitive nuclear DNA element (GcpR1), originally from the bean anthracnose pathogen *C. lindemuthianum* (44), was used successfully to differentiate among 10 species of *Colletotrichum* by producing unique polymorphic patterns for each representative species (Fig. 6A). When GcpR1 was used to compare *C. gloeosporioides* isolates from almond and avocado in Israel, the band patterns of the almond isolates were uniform and distinct from those of avocado isolates (17). Within the avocado isolates, polymorphic fragments were observed in accordance with the morphologic and VCG heterogeneity of this population.

C. Analysis of A+T-rich DNA associated with mitochondrial (mt)DNA. A+T-rich DNA, associated with the mitochondrial (mt) genome, can be detected by the restriction enzyme *Hae*III, which recognizes and cleaves the DNA sequence GGCC. A+T-rich DNA is cleaved infrequently by *Hae*III; whereas most of the nuclear DNA is digested to fragments of less than 2 kb in

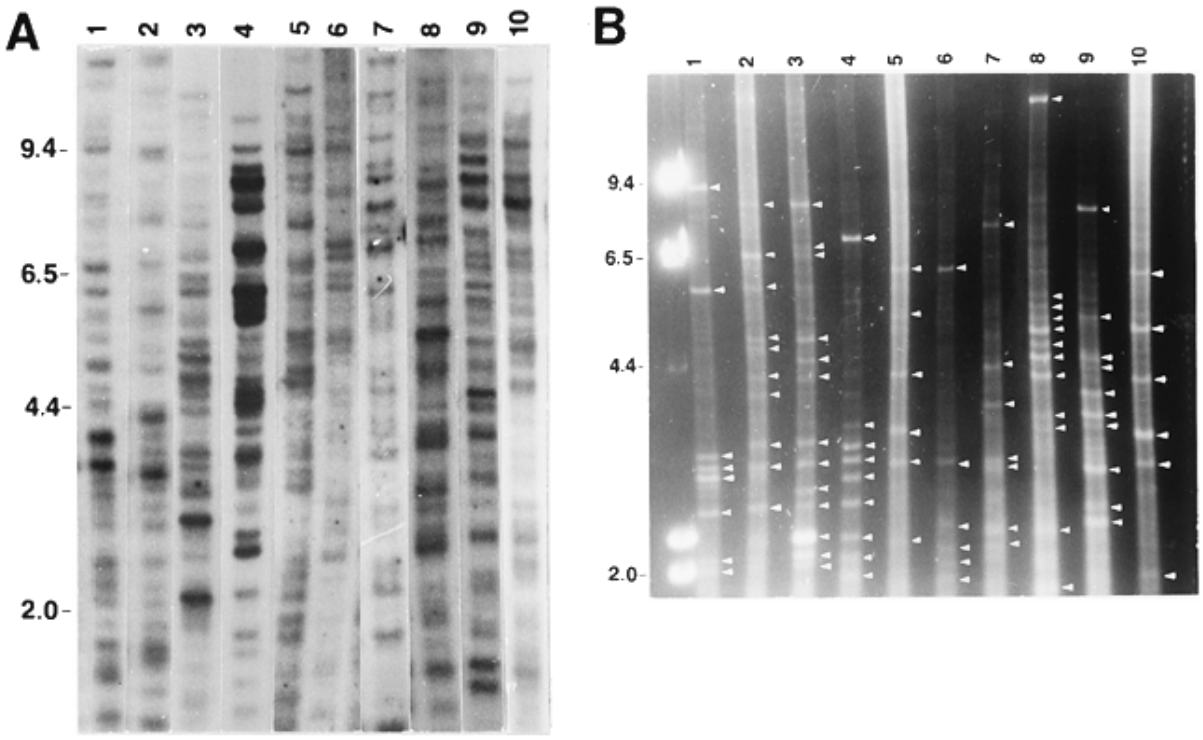

Fig. 6. Interspecies restriction fragment length polymorphism (RFLP) band patterns of DNA of 10 different *Colletotrichum* species, (A) digested with *Pst*I and hybridized with GcpR1 for nuclear DNA analyses, and (B) digested with *Hae*III for A+T-rich DNA analyses. Lane 1, *C. gloeosporioides* (isolate 231-1); 2, *C. musae* (isolate 927); 3, *C. fragariae* (isolate MS9-1); 4, *C. acutatum* (isolate 215-1); 5, *C. orbiculare* (isolate 15093); 6, *C. coccodes* (isolate 401); 7, *C. magna* (isolate GM-16); 8, *C. graminicola* (maize, isolate 102); 9, *C. graminicola* (sorghum, isolate TX-2536); 10, *C. lindemuthianum* (isolate NY-54). DNA markers with the sizes in kilobases are indicated to the left. Arrows in B indicate the bands chosen for pair-wise comparisons between species. (Figure courtesy Academic Press [19]).

size (19). *Hae*III RFLPs of A+T-rich DNA were used successfully to characterize *Colletotrichum* isolates from 10 species whereby each representative revealed unique restriction patterns (Fig. 6B). The results of this approach were similar to those obtained using the GcpR1 repetitive element, whereby *C. gloeosporioides* isolates from almond had unique band patterns, in contrast with polymorphic fragments observed for avocado isolates (17). This approach also allowed grouping of *C. acutatum* isolates from apple and peach and differentiation among *C. gloeosporioides* isolates from almond, apple, avocado, and mango (21).

D. ap-PCR. ap-PCR or random amplified polymorphic DNA (RAPD) has been used extensively for identification and characterization of isolates in *Colletotrichum* (2,12,53,54). We employed ap-PCR, using primers derived from minisatellite or repeat sequences (CAGCAGCAGCAGCAG, TGTCTGTCTGTCTGTC, GACACGACACGACAC, GACAGACAGACAGACA), for comparing *Colletotrichum* species from almond, avocado, strawberry, and other fruits. Unique band patterns were observed among the clonal populations of *C. acutatum* from strawberry and *C. gloeosporioides* from almond; whereas avocado isolates exhibited polymorphisms. ap-PCR was used to accurately and reliably differentiate among *C. acutatum, C. gloeosporioides,* and *C. fragariae* from strawberry (16,20): *C. acutatum* and *C. fragariae* populations shared a common band pattern, and the patterns for *C. gloeosporioides* were polymorphic. This method was useful in taxonomic identification of strawberry isolates from South Carolina as *C. acutatum* based on unique amplification products, in determining the uniformity of Israeli *C. gloeosporioides* isolates from almond, and in demonstrating heterogeneity within the species *C. gloeosporioides* in general, as illustrated by multiple band patterns (Fig. 7). Varying band patterns indicate that *C. acutatum* isolates from various hosts, including anemone, almond, apple, peach, pecan, and strawberry, cumulatively represent three genotypes, and that the overall heterogeneity is apparent within this species (Fig. 8).

In summary, rDNA analysis is a reliable method for taxonomic species identification; whereas analysis using GcpR1 and other repetitive DNA elements, A+T-rich DNA RFLPs, and ap-PCR are useful methods for determining subpopulation diversity within species.

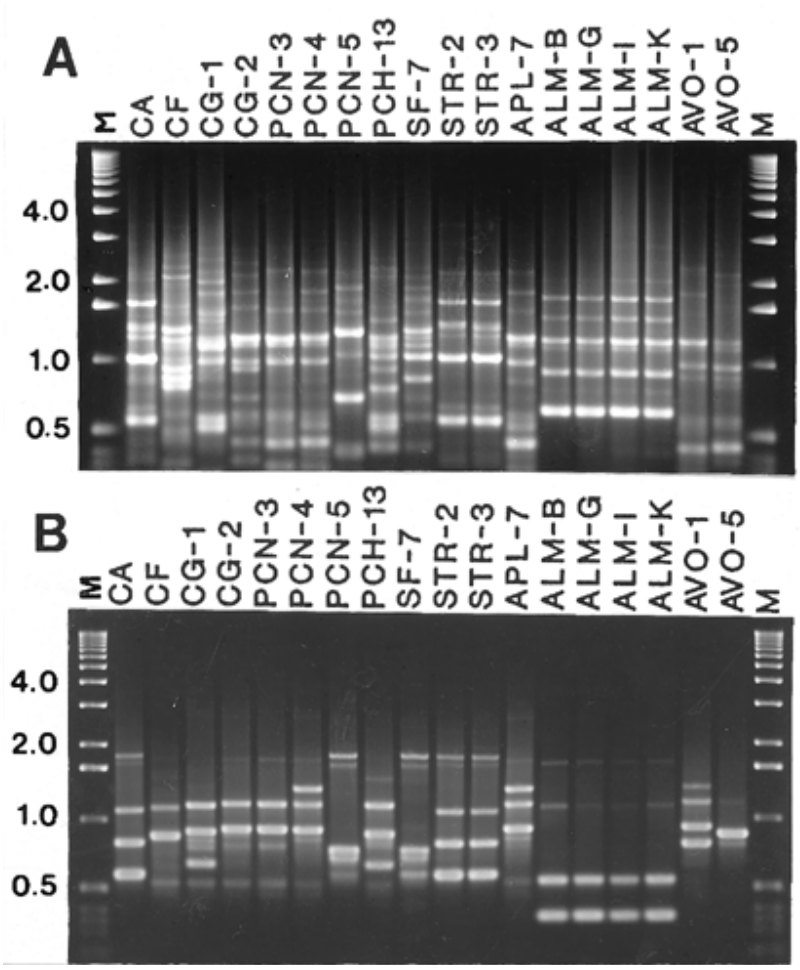

Fig. 7. Interspecies band patterns of arbitrarily primed polymerase chain reaction (ap-PCR) amplified genomic DNA from isolates of *Colletotrichum* from various hosts using primers (A) $(CAG)_5$ and (B) $(GACA)_4$ represented as follows: CA (*C. acutatum* from strawberry, California); CF (*C. fragariae* from strawberry, Mississippi); CG-1 and CG-2 (*C. gloeosporioides* from strawberry, Florida); PCN-3 and PCN-4 (*C. gloeosporioides* from pecan, Louisiana); PCN-5 (*C. acutatum* from pecan, Alabama); PCH-13 (*C. gloeosporioides* from peach, S. Carolina); SF-7 (*C. acutatum* from peach, S. Carolina); STR-2 and STR-3 (*C. acutatum* from strawberry, S. Carolina); APL-7 (*C. gloeosporioides* from apple, N. Carolina); ALM-B, ALM-G, ALM-I, and ALM-K (*C. gloeosporioides* from almond, Israel); and AVO-1 and AVO-5 (*C. gloeosporioides* from avocado, Israel). Lanes M contain DNA size markers in kilobases.

Effect of Teleomorphism on Genetic Diversity

Although *C. gloeosporioides* infects hundreds of different plant species (56), individual strains may have restricted host ranges, as demonstrated by the clonal subpopulation of *C. gloeosporioides* from almond grown in Israel (17,33). It is often presumed that host-specific populations reproduce only asexually, although certain isolates of *C. gloeosporioides* with different host specificities mate under laboratory conditions (10). Highly variable populations are more adaptable to changing conditions than are those with little variation, and sexual reproduction and recombination is a major factor that contributes to the genetic variability observed in fungal populations. *C. gloeosporioides* is a highly variable species, as shown by morphological characters and molecular markers. As mentioned above, *C. gloeosporioides* from avocado is heterogeneous and genetically complex (17). Furthermore, it is known that some strains of *C. gloeosporioides*/*G. cingulata* from avocado produce the sexual stage in culture (48; T. Katan, *unpublished*) and therefore may also reproduce via their *G. cingulata* teleomorph stage in the orchard. These findings are in contrast with the uniform genotype and single VCGs found among *C. gloeosporioides* from almond and *C. acutatum* from strawberry, which are strictly vegetatively reproducing clonal populations (16,17,33). The data suggest that recombination of vegetative incompatibility and other loci that occurs during sexual reproduction results in a high degree of genetic variation among *C. gloeosporioides* strains on avocado. Alternatively, the population may consist of numerous vegetatively (asexually) reproducing clones. These two reproduction strategies are not mutually exclusive and may coexist.

Summary

Colletotrichum is a broad-range pathogen with cases of multiple species on a single host on the one hand (Table 1), and single species on diverse hosts on the other

(Table 2). In this article, we have emphasized that certain populations of *Colletotrichum* appear to be host-specific, for example *C. gloeosporioides* from almond in Israel. However, in other geographic regions the same host may be attacked by additional species, as recently demonstrated with *C. acutatum* causing almond anthracnose in California.

Numerous reports have determined that cross-infection potential exists among different species of *Colletotrichum* on a multitude of hosts. These studies were mainly conducted on detached plant material, such as fruit and leaves. Although artificial host inoculation is usually not reliable enough for assessing host specificity, it indicates the potential for infection. Our studies have emphasized this point, showing that *C. gloeosporioides* from almond grown in Israel was able to infect various fruits under artificial inoculation conditions, but isolations from naturally infected fruit provided no evidence that cross-infection had occurred under field conditions.

The fact that *Colletotrichum* species differ in their sensitivity to certain fungicides such as benomyl is critical for chemical pathogen control. This should be accounted for when implementing a spray program where a mixed population exists, such as *C. acutatum* and *C. gloeosporioides* in apple, citrus, mango, peach, pecan, and strawberries, since differential sensitivity may result in a shift in population ratio.

The use of traditional methods (morphology, colony color, conidia size, etc.) may not be accurate enough for identification of species and subspecies, which is critical for disease management, resistance breeding, and pathogen control. Vegetative compatibility and molecular approaches offer complementary means for characterization of species. Molecular approaches have been utilized successfully for taxonomic identification and determination of genetic diversity of *Colletotrichum* populations. In a number of recent studies, species-specific primers have been used to differentiate between *C. acutatum* and *C. gloeosporioides* from various hosts. In order to use this approach more successfully for pathogen diagnosis in vivo, it is necessary to develop simple and rapid DNA extraction techniques, to allow multiple sampling and testing. Where *Colletotrichum* is considered a quarantine pathogen, DNA can be transferred safely between countries for diagnostic purposes, thus avoiding pathogen dissemination.

Population diversity is hypothesized to stem primarily from sexual reproduction. Understanding and observing the sexual stage is important in determining whether a population is homogeneous or heterogeneous, and for developing a sexual genetic system. The use of classical morphology and molecular tools may contribute to the understanding of the complexity of certain populations, such as that of *C. gloeosporioides* from avocado, which produces the teleomorph in culture and may survive and proliferate in the same manner in nature.

Future Outlook

Many anthracnose-susceptible hosts are cultivated worldwide. Damage to yield is especially significant in the tropics, where multiple hosts such as mango, avocado, coffee, papaya, banana, and citrus are grown in close proximity. Therefore, the complexity related to host range and specificity in the genus *Colletotrichum* must be determined for each host at every given location.

Future research should attempt to determine host specificity of *Colletotrichum* species primarily according to natural infections rather than artificial inoculations. Artificial infections are easy to obtain on detached leaves and especially on ripe fruit under experimentally controlled conditions in the laboratory. But since such experiments often use high inoculum levels and optimized humidity and temperature, their results may not accurately reflect the true virulence potential.

The taxonomy of *Colletotrichum* species is in a state of constant flux and remains confusing. The situation is complicated in particular with the species *C. acutatum* and *C. gloeosporioides*, which attack several hosts. Due to their broad (sometimes overlapping) host ranges and phenotypic and genotypic heterogeneity, *C. acutatum* and *C. gloeosporioides* are considered cumulative species composed of diverse subpopulations. Although the traditional methods to classify these fungi into distinct species are based primarily on morphological criteria, deviation from type-culture characteristics and the presence of intermediate forms have introduced ambiguity into species identification. The validity of these mycotaxons as "mycological (formerly botanical) species" is beyond the scope of this article but seems to be significant from a phytopathological point of view.

Of the molecular approaches mentioned earlier, the use of species-specific primers for PCR amplification of unique rDNA fragments seems the most promising for differentiating among the species of *Colletotrichum.* Since PCR-based methods are relatively simple, this approach should be further developed by adjusting the available primers and designing new ones for unequivocal identification of all (problematic) species. The dependability of these primers should be validated by testing them with *Colletotrichum* strains representing diverse populations of each species.

Although subpopulations have been recognized within *C. acutatum* and *C. gloeosporioides,* their taxonomic status is unclear. From the phytopathologic viewpoint, the considerable pathogenic variation evident within these species (often coupled with additional phenotypic variation) may warrant further dissection into subspecific groups designed to reflect host specificity. Ideally, the species name should refer to a broad grouping, with strains from the subspecies level indicating host specialization. The general acceptability of such subgrouping may require additional support, based on biological criteria independent of pathogenicity. Molecular and VCG analyses are likely candidates to provide the necessary information.

Molecular approaches have been widely used, and their power for characterization

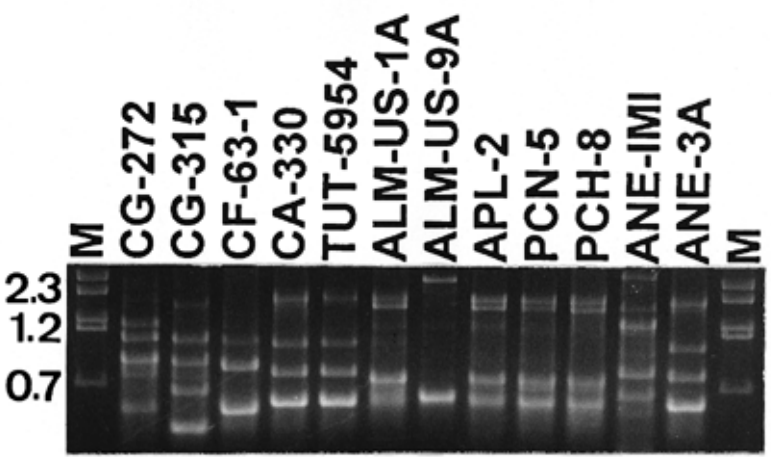

Fig. 8. Interspecies band patterns of arbitrarily primed polymerase chain reaction (ap-PCR) amplified genomic DNA from isolates of *Colletotrichum* from various hosts using primer $(GACA)_4$ represented as follows: CG-272 and CG-315 (*C. gloeosporioides* from strawberry, Florida and Nova Scotia); CF-63-1 (*C. fragariae* from strawberry, Mississippi); CA-330 (*C. acutatum* from strawberry, Tennessee); TUT-5954 (*C. acutatum* from strawberry, Israel); ALM-US-1A (*C. acutatum* from almond, California); ALM-US-9A (unidentified species from almond, California); APL-2 (*C. acutatum* from apple, S. Carolina); PCN-5 (*C. acutatum* from pecan, Alabama); PCH-8 (*C. acutatum* from peach, S. Carolina); ANE-IMI (*C. acutatum* IMI-223120 from anemone); and ANE-3A (*C. acutatum* from anemone, Israel). Lanes M contain DNA size markers in kilobases.

of isolates is well recognized. However, the applicability of the various techniques for overall delineation of subspecific subpopulations has yet to be determined. In particular, the capacity to accurately identify phenotypically similar strains from diverse sources and to separate them from other phenotypes by molecular markers must be demonstrated.

VCG analysis, commonly used to study formae speciales of *Fusarium oxysporum*, has only been applied in a few cases to determine population structure in *Colletotrichum* spp. Identification of species by this method is confined to populations that are vegetatively compatible with well-defined reference strains. Within species, subdivision by VCG has proven very dependable, as evidenced by correlation with genotypes and phenotypic traits. VCG determination is based on multi-locus controlled self/nonself discrimination between fungal strains, and its results are clear-cut in most cases. It is only applicable to anamorphic, clonally related populations. In sexually reproducing populations with a *Glomerella* stage, relationships can be determined by sexual compatibility, which is the ultimate criterion for delineating mating populations within a species. Unlike the DNA-based methods, tests based on VCG and sexual crosses employ living fungi. Consequently, strains to be compared may have to be moved from one geographic location to another, thus creating a risk of undesired pathogen dissemination.

Additional work should concentrate on trying to link molecular markers to biological traits, such as morphology, VCG, and virulence. There is no biological meaning to PCR-amplified bands from random primers that are unrelated to functional genes. However, RAPDs can still maintain their importance in a population genetics study at the subspecies level.

Additional areas of study should emerge with the recent observation of the teleomorph of *C. acutatum* from apple and blueberry in the southeastern United States (23). Multiple genotypes of *C. acutatum* exist as well as isolates of the species appearing on multiple hosts. Although clonal populations have been observed (e.g., on strawberries in Israel), it remains to be seen whether the presence of the sexual stage may significantly contribute to genetic diversity within *C. acutatum*, similarly to that hypothesized for *C. gloeosporioides.*

Stanley Freeman **Talma Katan** **Ezra Shabi**

Dr. Freeman is a senior researcher in the Department of Plant Pathology at the Agricultural Research Organization (ARO), Volcani Center. He received his B.S. in horticulture in 1982, and his M.S. in 1985 and Ph.D. in 1991 in plant pathology from the Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel. He was a visiting postdoctorate fellow supported by BARD during 1991 to 1993 at the University of California, Riverside, where he specialized in molecular fungal biology of the species *Colletotrichum.* He joined the Volcani Center in 1993. His research interests focus primarily on etiology, control, and fungus–host interactions of *Colletotrichum* and *Fusarium* species on vegetable and fruit crops.

Dr. Katan is a senior research scientist in the Department of Plant Pathology at the ARO. She received her B.S. and M.S. in genetics at the Hebrew University of Jerusalem and her Ph.D. in plant genetics at the Weizmann Institute of Science, Rehovot. She joined the ARO in 1977 as a fungal geneticist. Her research focused on fungicide resistance in fungal pathogens of fruit trees, vegetables, and field crops. Her current research interests, in addition to a continuing interest in fungicide resistance, focus primarily on vegetative compatibility grouping among populations of *Fusarium oxysporum* and *Verticillium* on vegetables, ornamentals, and field crops, and *Colletotrichum* on fruit trees, strawberry, and ornamentals.

Dr. Shabi is a senior research scientist in the Department of Plant Pathology at the ARO. He received his B.S. in agronomy in 1960, and his M.S. in 1962 and Ph.D. in 1979 in plant pathology from the Hebrew University of Jerusalem. In 1966, he joined the ARO as a researcher of deciduous fruit, grape, and olive diseases. He spent sabbatical leaves at the University of Missouri, Columbia (1974 to 1975); the University of California, Davis, and Cornell University NYSAES, Geneva, NY (1980); INRA, Bordeaux, France, Lincoln University, New Zealand, and Clemson University, SC (1991 to 1992); and HRI, Wellesbourne, UK (1996 to 1997). His work has focused on disease control, epidemiology, and resistance of fungi to fungicides.

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