The influence of climate on sexual reproduction in the Apple Scab Fungus *Venturia inaequalis* in Israel

Eric W.A. Boehm[1*], Stanley Freeman[2], Ezra Shabi[2] & Themis J. Michailides[1]

[1]Dept. of Plant Pathology, University of California Davis. [2]Dept. of Plant Pathology, ARO-Volcani Center, Bet Dagan, Israel. [*]Dept. of Biology, SUNY Potsdam.

Fig. 1. Map of Israel indicating the apple orchards sampled. Only the Golan Heights orchards (El Rom & Ortal) experience low winter temperatures.

The plant disease Apple Scab, caused by the ascomycete fungus *Venturia inaequalis* (Cke.) Wint. (anamorph *Spilocea pomi* Fr.), is the single most important disease of cultivated apple (*Malus domestica* Borkh.) worldwide. Severe apple crop losses can result when appropriate control measures are not taken, especially when the spring and early summer seasons are moist and cool. The fungal pathogen has a pronounced low temperature requirement for the initiation of the sexual or pseudothecial stage. In temperate apple growing regions of the world, this temperature requirement is met only after leaf fall, on the orchard floor, where, in winter months, low temperatures trigger the development of pseudothecia. In the spring, overwintered pseudothecia time their production of ascospores to precisely coincide with the emergence of new leaves, thus initiating a new cycle of disease.

In Israel, apple production can be divided into two ecological zones, characterized primarily by the presence or absence of low winter temperatures due to elevation: the Golan Heights (600 – 1300 m above sea level) and the Hula Valley (100 m above sea level) & the coastal plain. Only on the Golan Heights are sustained low winter temperatures present, whereas such temperatures are rare in the Hula Valley and non-existent along the coastal plain. Our hypothesis was that this intrinsic difference in Israeli apple ecology has had a direct impact on the life cycle of the pathogen, attenuating the importance of the sexual stage in low elevation apple production areas. Since sexual reproduction in this fungus has an obligate cold requirement for sustained low winter temperatures, and since these requirements in Israel are met only on the Golan Heights, we were interested in whether lower elevation populations might be comprised of asexual clonal lineages. This would have bearing on control strategies for the disease in Israel and may impact on the propensity of this pathogen to develop fungicide resistance.

The present study[1] was initiated to determine whether differences in genotypic diversity among populations of *V. inaequalis*, as detected using neutral genetic markers associated with microsatellites, were related to the ecological conditions in which apples are grown in Israel. Microsatellites are short (5 – 30bp) repeated sequences found throughout the eukaryotic genome. Since they are non-coding and under little constraint to maintain sequence conservancy, they evolve quite rapidly and provide useful genetic markers to monitor individuals in populations. In this study, genotypic diversity was measured using PCR & oligonucleotide primers designed to fungal microsatellites under high annealing temperatures (microsatellite primed or MP-PCR). Two orchards were sampled from the Golan Heights (El Rom & Ortal; n = 38) and three orchards from the Hula Valley and coastal plain ((Sede Eliezer, Ginaton and Be'er Tuvia; n = 40). MP-PCR data was analyzed as combined binary data sets using both cluster (UPGMA in NTSYS-pc Version 2.1) and parsimony (PAUP Version 4.0b4a) analysis.

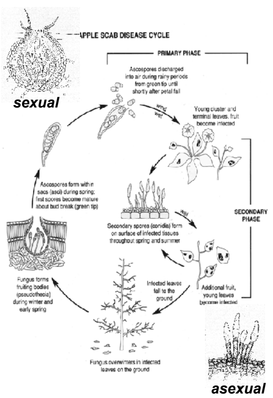
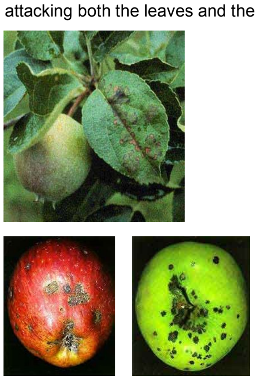


Fig. 2. (above) The life cycle of the Apple Scab fungus *Venturia inaequalis*. Low winter temperatures are required to trigger the sexual stage which initiates the disease in the spring. The asexual stage provides inoculum to spread the disease during the summer months.

Fig. 3. (below). Typical Apple Scab symptoms. The fungus is capable of attacking both the leaves and the fruit.



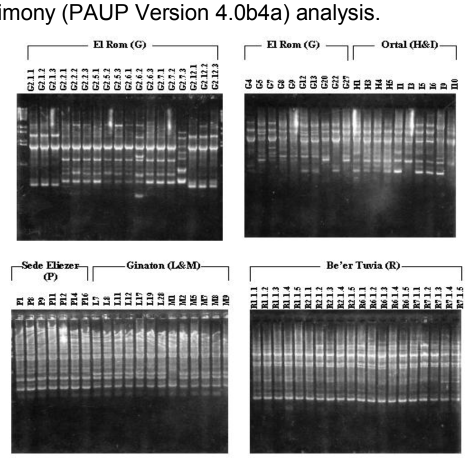


Fig. 4. Ethidium bromide stained MP-PCR gels of 78 Israeli isolates of *Venturia inaequalis* collected from the Golan Heights (El Rom, Ortal), the Hula Valley (Sede Eliezer) & the coastal plain (Ginaton, Be'er Tuvia) amplified with the microsatellite primer $(GACAC)_3$. Populations from the coastal plain were genetically uniform within each of the orchards sampled (P, L, M & R), indicating asexual clonal lineages. Whereas populations from the Golan Heights (G, H & I) showed levels of genotypic diversity ten times as high. The 18 El Rom isolates in the first gel (top left) all originated from multiple lesions along a *single apple leaf*. All other isolates each originated from a different tree in the orchard.

TABLE 2. Arithmetic means of genetic distance[z] within (*italicized*) and between geographic groupings of Israeli *Venturia inaequalis* populations

	G[y] n=10	H/I n=10	P n=7	L/M n=13	R[y] n=4
G: El Rom	*0.3477*				
H/I: Ortal	0.4246	*0.1579*			
P: Sede Eliezer	0.3930	0.4213	*0.0114*		
L/M: Ginaton	0.4174	0.4090	0.2149	*0.0*	
R: Be'er Tuvia	0.4840	0.4979	0.3428	0.2175	*0.0*

[z]Genetic similarities were computed for all pairs of isolates using the formula given by Nei (14): $S = 2b_{ij} / (b_i + b_j)$, where b_{ij} is the number of amplicons shared by two isolates, $_i$ and $_j$, and b_i and b_j are the total number of amplicons found in isolates $_i$ and $_j$, respectively. Genetic distances (dissimilarities) were calculated as 1 - S.

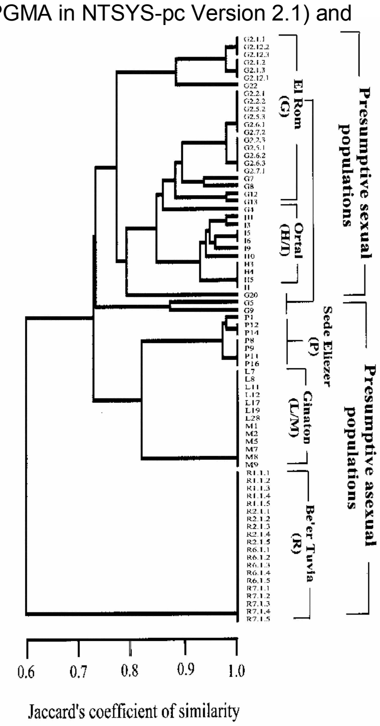


Fig. 5. Phenogram generated by cluster analysis (UPGMA in SAHN package of NTSYS-pc Version 2.1). Both cluster & parsimony (PAUP) analysis yielded similar hierarchical groupings & comparable levels of genetic diversity. Isolates of *V. inaequalis* sampled from the Golan Heights (El Rom & Ortal) were extremely genetic diverse. Whereas, asexual clonal lineages were observed from lower elevation apple productions areas that do not experience winter conditions. These data support field observations that this pathogen does not reproduce sexually in regions characterized by the absence of low winter temperatures. This is the first study to document asexual populations of this economically important plant pathogen and has bearing on control strategies for the disease in Israel.

Literature:

[1] Boehm E.W.A., Freeman, S., Shabi, E. & T.J. Michailides. 2003. Microsatellite primers indicate the presence of asexual populations of *Venturia inaequalis* in coastal Israeli apple orchards. *Phytoparasitica* 31(3): 236-251.