ABIOTIC VERSUS BIOTIC PATHOGENS: REPLICATIVE GROWTH IN HOST TISSUES KEY TO DISCRIMINATING BETWEEN BIOTOXIC INJURY AND ACTIVE PATHOGENESIS. Andrew C. Schuerger[1], Douglas W. Ming[2], and D. C. Golden[2]. [1]University of Florida, Bldg. M6-1025, Space Life Sciences Lab, Kennedy Space Center, FL 32899; email: schuerg@ufl.edu; [2]Astromaterials Research and Exploration Science Office, Mail Code JE23, NASA JSC, Houston, TX 77058; emails douglas.w.ming@nasa.gov and d.c.golden1@nasa.gov, respectively,

Introduction. Life can be defined as a self-sustaining chemical system capable of undergoing Darwinian evolution; a self-bounded, self-replicating, and self-perpetuating entity [1]. This definition should hold for terrestrial as well as extraterrestrial life-forms. Although, it is reasonable to expect that a Mars life-form would be more adaptable to Mars-like conditions than to Earth-like environments, it remains possible that negative ecological or host interactions might occur if Mars microbiota were to be inadvertently released into the terrestrial environment.

A biogenic infectious agent can be defined as a self-sustaining chemical system capable of undergoing Darwinian evolution and derives its sustenance from a living cell or from the by-products of cell death. Disease can be defined as the detrimental alteration of one or more ordered metabolic processes in a living host caused by the continued irritation of a primary causal factor or factors; disease is a dynamic process [2]. In contrast, an injury is due to an instantaneous event; injury is not a dynamic process [2]. A *causal agent of disease* is defined as a pathogen, and can be either abiotic or biotic in nature.

Diseases incited by biotic pathogens are the exceptions, not the norms, in terrestrial host-microbe interactions. Disease induction in a plant host can be conceptually characterized using the Disease Triangle (Fig. 1) in which disease occurs only when all host, pathogen, and environmental factors that contribute to the development of disease are within conducive ranges for a necessary minimum period of time. For example, plant infection and disease caused by the wheat leaf rust fungus, *Puccinia recondita*, occur only if virulent spores adhere to genetically susceptible host tissues for at least 4-6 hours under favorable conditions of temperature and moisture [3]. As long as one or more conditions required for disease initiation are not available, disease symptoms will not develop.

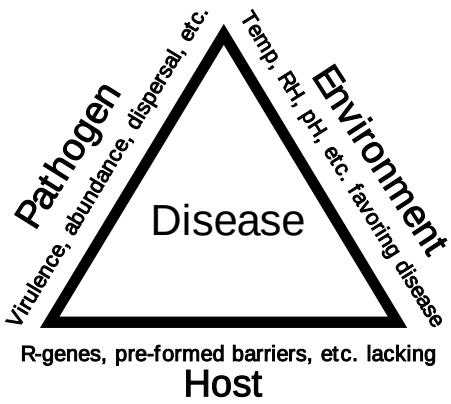


Figure 1. Disease Triangle.

Life Detection in Mars Samples. In order to release returned Mars samples to the general scientific community, several criteria must be met that convince the community at large of the safety of the materials. The following sequence is proposed as a preliminary experimental structure to determine the biosafety of returned Mars samples.

First, terrestrial life is based on carbon, and it is likely that an extraterrestrial pathogen also must be based on carbon if it is to gain sustenance from terrestrial life-forms. If true, then analyses of martian regolith and rocks for organics will be the first line of defense in assaying returned samples for the presence of potential pathogens. Second, a series of replicative assays should be conducted in culture (i.e., outside host tissue) to determine if putative Mars microbiota are present in the samples and capable of growth and cellular replication under a diversity of environmental conditions spanning the range from the martian surface to terrestrial ecosystems. And third, bioassays should be conducted with plant, animal, invertebrate, and microbial systems to confirm the absence of harmful biological entities in the samples. If all three of these tests are negative, the samples are likely to be safe to release to the community.

However, to date, there are no established protocols for assaying returned samples to demonstrate their biosafety to terrestrial life-forms or ecosystems. The primary objective of this project was to investigate the effects of aqueous extracts of Mars analog soils on the plant host *Capsicum annuum* (pepper; a traditional host indicator crop for viral, bacterial, and fungal pathogens) in order to begin the development of protocols that might discriminate between abiotic (not a safety issue) versus biotic pathogens.

Materials and Methods. Six Mars analog soils were generated from terrestrial minerals, crushed and sieved to pass 500 µm stainless steel sieves, and stored at 24 C until used. The six Mars soils were created to represent: (1) a benign basalt-only soil, (2) high-salt soil, (3) acidic soil, (4) alkaline soil, (5) perchlorate soil, and (6) an aeolian dust simulant. Aqueous extracts of each simulant were created by vigorously shaking 50 g of soil in 100 ml of sterile deionized water (SDIW; 18 Ω) for 2 h in a baffled 250 ml flask. The extracts were filtered through Whatman #4 paper, 0.45 µm, and 0.22 µm filters. The pH and electrical conductivity (EC) of analog soils are given in Table 1.

Table 1. pH and EC for six Mars analog soils.

Analog soils	pH	EC
Basalt (control)	8.1	68.2 µS cm^{-1}
High salt	2.9	18.3 mS cm^{-1}
Acidic	2.7	38.8 mS cm^{-1}
Alkaline	10.2	11.6 mS cm^{-1}
Phoenix	6.7	5.5 mS cm^{-1}
Aeolian dust	6.7	6.9 mS cm^{-1}

The aqueous extracts (0.5 ml/injection) of the Mars simulants were then injected into leaves of 28-d old pepper plants (*C. annuum*, cv. Hungarian Wax) using 20G needles (Fig. 2). The procedure is used as a standard method of injecting fluids with presumptive pathogens into leaf tissues in order to screen for microbial virulence. For viral pathogens, pepper leaves can exhibit local lesions (e.g., Fig. 3, tomato mosaic virus [ToMV] on tobacco), leaf chlorosis (yellowing), veinal collapse, systemic development of symptoms, and eventual necrosis of both leaf and canopy tissues. For bacterial pathogens, a water soaked lesion around the point of injection is typically observed within a few days. For fungal pathogens, the response generally begins with chlorosis near the injection followed by production of macroscopic reproductive structures on or in leaf tissues. Leaf and plant wilt is possible with all three classes of biological pathogens.

Fig. 2

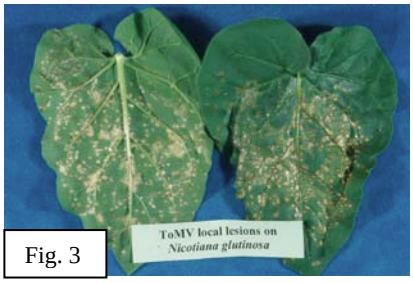


Fig. 3

Results. Injection of SDIW (black marks in Figs 4 and 5) produced no discernable symptoms in pepper leaves. The SDIW was simply reabsorbed by the leaf tissues, and leaves appeared normal within 2-4 h (Fig. 4). In contrast, injections of the high-salt simulant extracts induced interveinal chlorosis on the treated leaf, followed by tissue necrosis at the points of fluid injection. Acidic soil extracts induced rapid necrosis of the fluid saturated injection sites (within 2 h) and total-leaf necrosis within 48 h. However, if the symptomatic tissues for both salt and acid simulants were ground in SDIW in sterile mortars and pestles, and then injected into healthy and symptom-free pepper leaves, no additional symptoms were observed.

In a separate series of tests, high salt and [illegible] simulant extracts were titrated to pH 7, and then 0.5 ml injected into fresh symptom-free pepper leaves. Results for the acid and salt aqueous extracts (e.g., Fig. 5; high-salt simulant) indicated that necrosis was observed for the low-pH soil extracts, but was absent for the pH 7 titrated simulant extracts. Results suggest that the primary edaphic factor that was responsible for leaf necrosis was low pH in both soil extracts (Table 1).

Fig. 4

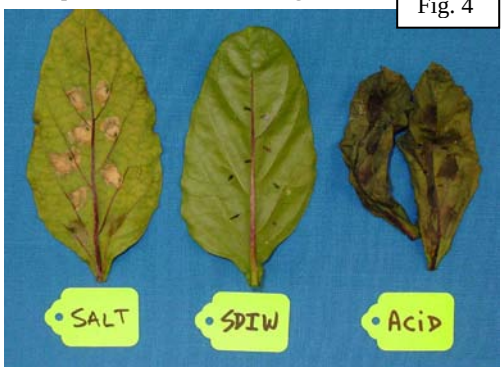
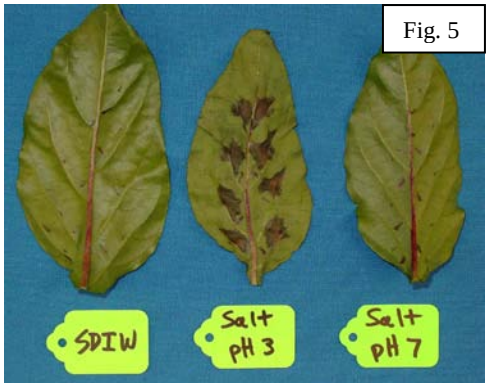


Fig. 5



Results from leaf injections of other soil extracts indicated minor biotoxic responses in pepper leaves for neutral pH extracts from the perchlorate and aeolian dust simulants. The high pH alkaline soil extracts induced symptoms similar to the aqueous extracts from the salt and acidic simulants, but to a lesser degree than salt or acid soil extracts. In all cases, if symptomatic leaf tissues from the 1st set of injections were ground in SDIW using sterile mortars and pestles, and then injected into healthy pepper leaves, tissues failed to develop any of the symptoms described above.

Conclusions. Bioassays are proposed here as an essential part of assessing the biosafety of returned samples from Mars. Due to the extremes of pH, EC, or other edaphic factors that are likely to be present in some Mars samples, biotoxic injuries of challenged tissues are likely. As abiotic factors are diluted through subsequent challenges, symptoms of biotoxic injury are likely to disappear quickly through time due to dilution. In contrast, a biological pathogen capable of growth and replication through multiple generations is likely to repeatedly induce disease symptoms over multiple challenges. For example, the local lesions induced by ToMV in tobacco (Fig. 3) would continue to induce new local lesions indefinitely over multiple generations. Thus, replicative growth of a presumptive microbial pathogen from Mars might induce symptoms that can be differentiated from one-time injury effects by edaphic factors in samples. If symptoms persist over time and through multiple sequential challenges, a biological entity must be assumed to be present, even if replicative evidence in culture (i.e., free of host tissue) is lacking.

References [1] Chyba C. F. and McDonald G. D. (1995) Ann. Rev. Earth Planet. Sci. 23:215-249; [2] Bateman D. F. (1978) The Dynamic Nature of Disease, Pages 53-83 In: Plant Disease,Vol. III, eds. Horsfall J. G. and Cowling E. B., Academic Press, New York; [3] Vallavieille-Pope et al., (1995) Phytopathology 85:409-415.