

Critical Parameters for Predictive Modeling of Chemical Agent Persistence on the Battlefield: The Effective Half-Life of Chemical Agent on Plant Foliage

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Abstract

Methods for investigating chemical agent-plant interactions have successfully been developed using live plant species, necessary in order to maintain plant physiological responses and obtain results applicable to agent contaminated battlefields. Pioneering research was undertaken, sustaining living plants within mandatory surety hood constraints and disseminating agent onto individual mature leaves of the foliage of intact living plants. The primary plant species selected for method development and initial research investigation is the grass *Echinochloa crus-galli*. Grass is the most prevalent type of plant worldwide, and the natural distribution of *Echinochloa crus-galli* is one of the largest for grass plants worldwide.

Plant culture methods for growing and maintaining grass within a surety hood environment included the establishment of appropriate growth media and nutrition, moisture regime and maintenance, germination rate, time-to-mature leaf stage, and the testing and installing of a system of light-emitting diodes (LED) within the surety hood in order to supply high-quality photosynthetically active radiation (PAR) without the usual concomitant excessive heat load of traditional illumination. Traditional plant culture under controlled measurable environmental conditions outside of surety hoods typically involves balancing heat loads with large chilling units that are too cumbersome for functioning surety hoods. However, physiologically healthy living plants are required in order to investigate and record critical parameters for the effects of agent-plant interaction, rather than creating and observing artifacts from the impact of factors other than warfare agent.

The preliminary result observed for the effective half-life of VX [O-Ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate] on grass leaves (illumination >250 $\mu\text{moles cm}^{-2} \text{sec}^{-1}$, 16h light / 8h dark; 21°C; relative humidity >45%) is 59 hours (± 14 asymptotic standard error; 95% Confidence Limits 30-88), calculated using the statistical Logistic Gompertz Model that provided the best fit to the observed experimental data. The effective half-life is a measure, as a function of time post-dissemination, of the net effect of all factors affecting the persistence of the agent, including agent evaporation, transformation, and fixation.

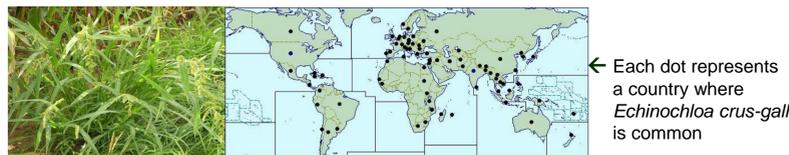
Additional critical parameters under current investigation include the coefficient of wash-off from measured rainfall, distribution of agent on and within leaves as a function of time post-dissemination, and contact transfer (exposure) of agent from contaminated foliar surfaces onto Army Combat Uniforms (ACU). Results of these investigations provide critical parameter input for predictive models, and direct experimental determinations for comparison of predictive model outcomes.

Background Information

- Little information existed on the interactions of chemical warfare agents (CWA) on plants.
- New methods needed to be developed for sustaining plant culture in a Surety Hood environment, in order to investigate chemical agent-plant Interactions using healthy live plant species with phytophysiological responses applicable to contaminated battlefields.
- CWA on foliage has to be within an appropriate range (e.g., battlefield), yet be detectable.

Materials and Methods

Plant species: *Echinochloa crus-galli* (L.) P. Beauv., a species of grass; Grass is the most prevalent type of plant worldwide, and the natural distribution of *Echinochloa crus-galli* is one of the largest worldwide^{1,2}



Physiology: Mature leaf stage

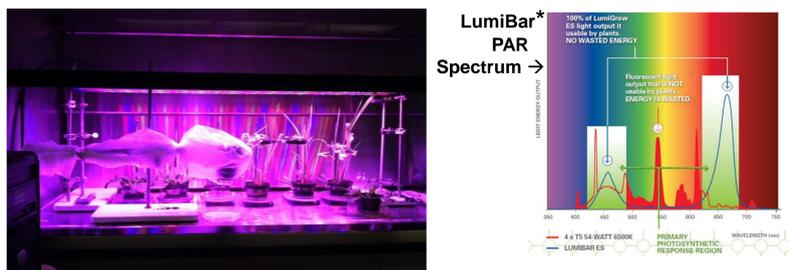
CWA: VX [O-Ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate]

Dissemination: 1 μL VX droplet (near optimal range for material testing³) onto surface of individual mature leaf, horizontally stabilized; randomly selected mature plant leaves received treatment; quadruplicate replicates

Sample Times: Individual leaves that received VX, and control leaves, were collected at 0.017, 1, 4, 24, 48, 120, 168 hours after VX dissemination

Plant culture: (pre-surety hood) Twenty seeds are sown in potting mix, and hydrated with ASTM Type I water (18 M Ω cm); following germination (80-95% after 7-10 days), individual grass plants are transplanted into 100mm containers and randomized within an environment-controlled plant growth chamber; Plants thereafter receive dilute aqueous nutrient solution every 2-3 days to maintain moisture (by mass) and sustain healthy plants. Chamber conditions: 22°C \pm 2 (16h-light) and 18°C \pm 2 (8h-dark); relative humidity 60% \pm 5; illumination 300-350 $\mu\text{mol s}^{-1} \text{m}^{-2}$ photosynthetically active radiation (PAR; 400-700nm)

Plant culture: (surety hood) Plants with 2-3 fully-mature leaves (18-21 days after transplantation) are transferred into surety hood conditions: 21°C \pm 2; 16h-light and 8h-dark; relative humidity \geq 45%; illumination 300-350 $\mu\text{mol s}^{-1} \text{m}^{-2}$ PAR delivered using LumiGrow LumiBar LED Strip Lighting*:



* Mention of a product or Trade Name does not constitute endorsement

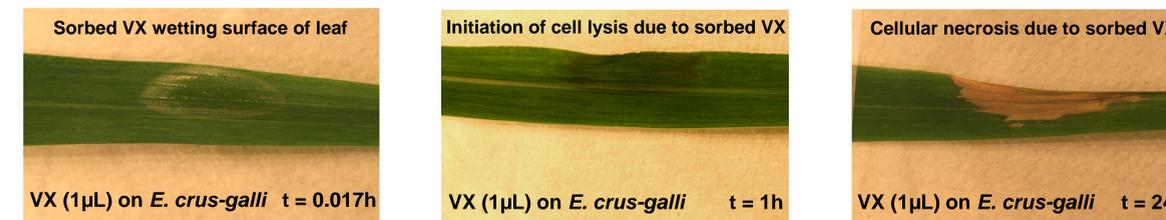
Materials and Methods (cont'd)

Leaf Analysis: Treated and control leaves are removed from plant, 14cm from the leaf tip, then prepared and analyzed by:

- 1) Placing each leaf into a Mylar[®] sample bag attached to a test tube
- 2) Flash-freezing in liquid N₂
- 3) Pulverizing leaf in Cryo-Prep Impactor
- 4) Adding extraction solvent
- 5) Focused Ultra-Sonication
- 6) Centrifugation
- 7) Analytical determination of VX by UHPLC-Triple Quad MS

Results

VX on leaf: Upon dissemination, VX spread and sorbed onto and into live mature grass leaves, wetting the leaf surface; After 1 hour the area where VX sorbed appeared darkened apparently due to initiation of cell lysis, then necrosis:



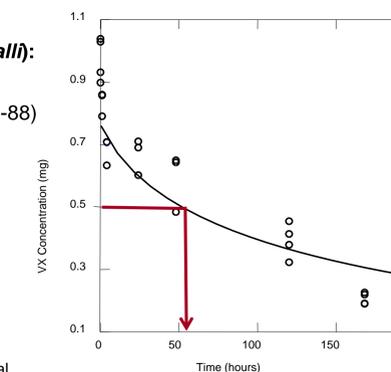
Data Analysis: The Logistic Gompertz Model⁴ provided the best fit to the observed experimental data

Preliminary result for Effective Half-Life of VX on Grass (*Echinochloa crus-galli*):
59 hours
(± 14 asymptotic standard error; 95% Confidence Limits 30-88)

The effective half-life is a measure, as a function of time post-dissemination, of the net effect of all factors affecting the persistence of the agent, including agent evaporation, transformation, and fixation

References

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