Current Topics in
Phytochemistry
Vol. 11, 2012

Original Communication

Captive bubble and sessile drop surface characterization of a submerged aquatic plant, *Hydrilla verticillata*

Christopher A. Dunlap[1,*], Girma Biresaw[2] and Judy F. Shearer[3]

[‡]USDA, Agricultural Research Service, National Center for Agriculturral Utilization Research,
[1]Crop Bioprotection and [2]Bio-Oils Research Units, 1815 N. University St., Peoria, IL 61604
[3]US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS 39180, USA

ABSTRACT

The surface energy parameters of the invasive aquatic weed, *Hydrilla vertcillata,* were determined using contact angle measurements using two different methods. The abaxial and adaxial surfaces of the leaves and stem were characterized for the weed while submerged in water using captive air and octane bubbles. For comparison, the adaxial surface of air-dried leaves was characterized using sessile drops of three liquids. The results of captive bubble experiments estimate the surface energy parameters to be similar for each of the plant components with a surface free energy of ~45 dyn/cm with polar and dispersive contributions approximately equal. Air-dried leaf surfaces had an estimated surface free energy of ~47 dyn/cm with a dispersive component of 38 dyn/cm and a polar component of ~9 dyn/cm. These results highlight the need to take into account the hydration status of these types of surfaces when analyzing their surface energy components. This report is the first to characterize the surfaces of this important weed and of submerged plant components in general.

KEYWORDS: *Hydrilla verticillata*, surface energy, aquatic plant contact angle, leaf

INTRODUCTION

Hydrilla vertcillata, is a submerged aquatic plant native to southeast Asia. In the rest of the world, it is an aggressive invasive species on every continent, except Antarctica. It is considered to be one of the most problematic invasive aquatic plants in the United States [1]. Introduced into Florida in the 1950's this invasive aquatic macrophyte is now found in lakes, ponds, reservoirs, rivers, and canals across southern United States, the Atlantic coastal states from Florida to Maine and from California to Washington on the Pacific coast. The plant is an excellent competitor in aquatic habitats because it can photosynthesize at low light levels, is insensitive to water quality, and produces several types of propagules [2].

When *H. vertcillata* growth reaches nuisance levels in water bodies it can cause significant economic damage by degrading habitat, impeding navigation, clogging irrigation and drainage ditches, and increasing both flooding and sedimentation [3]. Chemical control has been the treatment of choice for large-scale *H. vertcillata* management and fluridone has historically been the chemical of choice [3]. Fluridone is effective at use rates much lower than contact herbicides and is lower in treatment costs [4]. This has led to extensive use of fluridone in many lakes and reservoirs for management of *H. vertcillata*. This intensive use of fluridone has resulted in the development of fluoridone-resistant varieties of this weed in the United States [5], which requires the development of new management tools to control *H. vertcillata*.

---

*christopher.dunlap@ars.usda.gov

Our laboratories have been actively involved in developing a biological control option using a native plant pathogen isolated from diseased *H. vertcillata* [6]. The plant pathogen, *Mycoleptodiscus terrestris*, has been shown to be effective in controlling the weed under laboratory conditions [7]. Our laboratories have developed a culturing method of producing stable infective propagules of the fungus known as microsclerotia [8]. These microsclerotia are on the scale of 200-600 µm in size and are oval in shape. The size and shape of the propagule create an application challenge in getting them to stay and adhere on the plant surface long enough for the propagule to germinate. Developing a formulation that would promote adhesion of the propagule to the plant surface requires basic knowledge of the surface properties of the plant. To our knowledge, this is the first report of characterizing the physico-chemical surface properties of *H. vertcillata* or any submerged aquatic plant. This manuscript reports the characterization of the surface and interfacial free energies of the leaves and stem of *H. vertcillata*. We utilize captive bubble and the geometric mean equation [9] to characterize submerged parts. In addition, we utilized sessile drop techniques and several surface energy models to determine the surface energy parameters of air-dried leaves.

MATERIALS AND METHODS

Materials

The water used in this work was obtained by purifying deionized water to resistivity of greater than 18.0 megaohms-cm with an EASYpure UV/UF water purification system (Barnstead International, Dubuque, IA). The other probe liquids were obtained commercially and used as supplied. These were: diiodomethane, DM (99%, Sigma-Aldrich, St. Louis, MO); Octane (99%, Sigma-Aldrich, St. Louis, MO); and Formamide, FA (≥99.5%, Fluka, Buchs, Switzerland). The air used in the captive bubble measurements was passed through a C_{18} solid-phase extraction filter (Grace inc, Deerfield, IL) prior to use.

Hydrilla

Hydrilla verticillata was provided from the culture tanks of the US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS. Plant samples were packaged in drenched paper towels for overnight delivery to the analysis laboratory at the National Center for Agricultural Utilization Research. The samples were stored wet at 4ºC until the time of analysis, less than 24 h after receipt of shipment. Samples were briefly rinsed with deionized water to remove any foreign material. Plant parts were selected from healthy appearing plants near areas of emerging new growth. Samples were separated from the plant with forceps and a scalpel.

Captive bubble measurements

Plant parts were attached to the stage of a sealed interfacial tension (IFT) chamber using double-sided adhesive tape (Macbond IB1190, MACtac, Stow, OH) and submerged in ultrapure water. The temperature was maintained at 20ºC. Air or octane was introduced using a 25 gauge blunt tip needle through a sealed syringe port on the bottom of the chamber. Contact angles were measured using axisymmetric drop shape analysis on a FTA 4000 Dynamic Contact Angle and Surface Tension Analyzer (First Ten Angstroms, Portsmouth, VA). Bubbles averaged about ~6.5 µl in volume with a base width ~1.5 mm. Octane droplets average ~4.2 µl with a base width of around ~1.7 mm. Images were acquired at a rate of 0.0667 sec/image, which allowed for 400 images to be captured during a total acquisition period of 26 sec. Upon completion of image acquisition, each image was automatically analyzed using a non-spherical fit, and the contact angle as a function of time was determined. The contact angle reported represents the average contact angle obtained between 1 and 2 s after the bubble/droplet made contact with the surface. The reported contact angle θ_{water} is derived from $180^{o} - \theta_{air}$., θ_{water} is typically $<90^{o}$ in captive bubble experiments. After data collection, a sample of the water in the chamber was assayed using pendant drop methods to confirm there was no leakage of surface active components from the plant to water. Reported values represent the average of a minimum of 12 replicates.

Sessile drop measurements

The leaves were secured to a microscope slide using double sided tape to provide a stable platform. The leaves were allowed to slowly dry for ~75-90 minutes in a Petri dish before performing measurements. This drying time was experimentally determined as providing the most consistent results (data not shown). Contact angles were measured using the FTA 200 Dynamic Contact Angle and Surface Tension Analyzer (First Ten Angstroms, Portsmouth, VA). The accuracy of the instrument was ascertained by measuring the contact angle of water on Teflon-coated aluminum foil (Bytac®) mounted on glass slides. The average and standard deviation of 5 such measurements was 103.9 ± 0.8°, which is within the reported [10] range of 98-112° for the contact angle of water on PTFE (polytetrafluoroethylene or Teflon). The probe liquid was dispensed until a pendant drop was hanging from the tip of the needle, and the image acquisition was manually triggered when the drop fell off. Following the trigger, images were acquired at a rate of 0.0667 sec/image, which allowed for 150 images to be captured during a total acquisition period of 10.005 sec. Upon completion of image acquisition, each image was automatically analyzed using a non-spherical fit, and the contact angle as a function of time was determined. The contact angle reported represents the average contact angle obtained between 0.07-0.2 s after the drop made contact with the surface. During this period the contact angles were relatively stable. A minimum of 10 replicate measurements were made for each liquid.

RESULTS

Estimation of surface energies of plant parts submerged in water using captive bubble measurements

Captive bubble measurements with air and octane were used to characterize the surfaces of *H. verticillata*. The adaxial (top) and abaxial (bottom) surfaces of the leaves as well as the stem were analyzed. Leaves were primarily sampled from areas of new growth on the plant. The results are reported in Table 1. The results show the air contact angle to be very similar and within a small standard deviation for each plant part, whereas the octane contact angle changed significantly and had a larger standard deviation.

Table 1. Air and octane contact angles on hydrilla leaves and stems submerged in water.

Adaxial (top)	
θ_{air}	48.0 ± 2.1
θ_{octane}	110.0 ± 5.3
Abaxial (bottom)	
θ_{air}	43.5 ± 2.8
θ_{octane}	105.1 ± 6.2
Stem	
θ_{air}	46.7 ± 2.9
θ_{octane}	131.6 ± 7.1

The measured captive bubble contact angles were used to calculate the surface free energy using the Owens-Wendt method (geometric mean) [11]. The geometric mean equation (1) can be used to estimate the interfacial free energy between two phases using the polar and dispersive components surface free energy [9]. As follows:

$$\gamma_{12} \approx \gamma_{1v} + \gamma_{2v} - 2\sqrt{\gamma_{1v}^{d}\gamma_{2v}^{d}} - 2\sqrt{\gamma_{1v}^{p}\gamma_{2v}^{p}} \qquad (1)$$

Where the superscripts refer to (d) dispersive and (p) polar components of surface free energy and the subscripts refer to the phases (1 and 2) and (v) vapor phase. The leaf (L) and water (w) interfacial free energy can be estimated with the appropriate substitutions (2), likewise for the leaf and octane (o) interfacial free energy (3).

$$\gamma_{Lw} \approx \gamma_{Lv} + \gamma_{wv} - 2\sqrt{\gamma_{Lv}^{d}\gamma_{wv}^{d}} - 2\sqrt{\gamma_{Lv}^{p}\gamma_{wv}^{p}} \qquad (2)$$

$$\gamma_{Lo} \approx \gamma_{Lv} + \gamma_{ov} - 2\sqrt{\gamma_{Lv}^{d}\gamma_{ov}^{d}} - 2\sqrt{\gamma_{Lv}^{p}\gamma_{ov}^{p}} \qquad (3)$$

For a captive bubble at the leaf-water interface, Young's equation provides;

$$\gamma_{Lv} - \gamma_{Lw} = \gamma_{wv}\cos\theta_{w} \qquad (4)$$

and for an octane drop at the leaf-water interface, Young's equation provides;

$$\gamma_{Lw} - \gamma_{Lo} = \gamma_{ow}\cos\theta_{o} \qquad (5)$$

The values of several of these parameters are known $\gamma_{wv} = 72.1$ dyn/cm, $\gamma_{wv}^{d} = 21.6$ dyn/cm, $\gamma_{wv}^{p} = \gamma_{ow} = 50.5$ dyn/cm, $\gamma_{ov} = \gamma_{ov}^{d} = 21.6$ dyn/cm and

$\gamma_{ov}^{p} = 0$ [12]. Combined with these values, (3) can be subtracted from (2) and substituted into (5) and rearranged to yield (6).

$$\gamma_{Lv}^{p} \approx 12.7(1 - \cos\theta_o)^2 \qquad (6)$$

To calculate γ_{Lv}^{d} , Equation (2) can be rearranged and substituted with (4) then solved. The calculated surface and interfacial free energies from the captive bubble measurements are reported in Table 2. The results identify no significant differences in the surface and interfacial free energies of the different hydrated hydrilla samples. The abaxial leaf surfaces had the lowest surface free energy of the components at 42.6 dyn/cm, while the adaxial leaf surface and stem had a surface free energy of 45.4 dyn/cm.

Table 2. Surface and interfacial free energies (dyn/cm) by captive bubble measurements and the Owens-Wendt method (geometric mean).

Adaxial (top)	
γ_{Lv}	45.4 ± 9.8
γ_{Lv}^{d}	22.5 ± 6.1
γ_{Lv}^{p}	22.9 ± 2.9
γ_{Lw}	5.0 ± 0.8
Abaxial (bottom)	
γ_{Lv}	42.6 ± 6.7
γ_{Lv}^{d}	22.4 ± 3.8
γ_{Lv}^{p}	20.2 ± 2.5
γ_{Lw}	6.5 ± 1.9
Stem	
γ_{Lv}	45.4 ± 7.8
γ_{Lv}^{d}	43.9 ± 5.8
γ_{Lv}^{p}	35.2 ± 6.5
γ_{Lw}	4.7 ± 0.9

Estimation of surface energies of air-dried Hydrilla leaves from the contact angle of sessile drop measurements

As an alternative method of determining the surface free energy of hyrdrilla leaves, sessile drop measurements were used to characterize the properties of the adaxial leaf surfaces of hydrilla after drying. Three probe liquids were used to characterize the leaves; water (WA), formamide (FA), diiodomethane (DM). The observed contact angles for the liquids are reported in Table 3.

Table 3. Sessile drop contact angles on the adaxial surface of dried hydrilla leaves.

θ_{water}	67.0 ± 5.4
$\theta_{\text{formamide}}$	42.5 ± 4.0
$\theta_{\text{diiodomethane}}$	59.8 ± 3.8

The surface free energy and the dispersive, polar and acid/base components of the surface energy was calculated using 3 different methods: the VCG method [13], the Owens-Wendt method (geometric mean, GM) [11] and the Wu method (harmonic mean, HM) [14]. The results for the dried leaf surfaces are reported in Table 4. Analysis of the contact angles of WA, FA and DM using the VCG procedure gave the acid (γ^{A}), base (γ^{B}), polar (γ^{AB}), and total (γ_{Lv}) surface energy components for the leaf surface. The VCG procedure gave a high γ^{B} and very low γ^{A} values for the leaf surface. This resulted in a polar surface energy component from the VCG method that was almost 20 times lower than that predicted by GM and HM methods. As a result, the γ_{Lv} of the leaves from the VCG method was lower by almost 10 dyn/cm from those predicted by the GM and HM methods (Table 4). The GM and HM methods provided a γ_{Lv} of 47.7 ± 2.8 and 53.6 ± 2.6 dyn/cm, respectively.

DISCUSSION

In the present investigation, two methods were used to characterize the surfaces of *H. vertcillata*. The captive bubble method permits the analysis of the surfaces in a fully hydrated environment, which is the normal habitat for this species (i.e., submerged in water). While the sessile drop method requires the surface to first be dried. The two methods lead to significantly different calculated surface free energy for the adaxial leaf surface and interfacial energy between the leaf adaxial surface and water. Observed differences in interfacial free energy between the two methods are typically explained by changes in the conformation of surface polymers induced with drying. When the leaves are

Table 4. Surface energies and their components of the adaxial surface of dried hydrilla leaves calculated from sessile drop contact angle measurements (dyn/cm).

Surface energy	Method[a,b]		
	VCG	GM	HM
γ_{Lv}^{A} (DM, WA, FA)[c]	0.002		
γ_{Lv}^{B} (DM, WA, FA)[c]	20.57		
γ_{Lv}^{AB} (DM, WA, FA)[c]	0.44		
γ_{Lv}^{p} (DM, WA)[c]		9.4 ± 2.7	14.6 ± 2.6
γ_{Lv}^{d} (DM)[c]	38.3 ± 2.0	38.3 ± 2.0	38.98 ±1.84
γ_{Lv} (DM, WA, FA)[c]	38.73		
γ_{Lv} (DM, WA)[c]		47.7 ± 2.8	53.6 ± 2.6
γ_{Lw} (DM, WA)[c]		18.3 ± 2.4	

[a] Method abbreviations are: GM, geometric mean; HM, harmonic mean; VCG, van Oss, Chaudhury, and Good;

[b] See ref [9, 13, 14] for details about these methods

[c] Probe liquids used in the estimation of the indicated surface energies: DM, diiodomethane, FA, formamide, WA, water.

submerged in water, polymers on the surface of the leaves interact with water in a manner that minimizes the interfacial free energy between the leaf and the liquid. This would entail conformational changes in the surface polymers to bring the hydrophilic components of the polymers in contact with the liquid phase. While hydrophobic regions of the surface polymers would be oriented away from the liquid phase.

The surface energy parameters determined here will be used to guide future research in developing adhesive formulations of our bioherbicide, *M. terrestris*. The technical challenges of developing adhesive formulations for highly hydrated environments are similar to those in developing mucoadhesive formulations for health applications. A variety of polymers have shown potential in improving mucoadhesion and will serve as potential candidates to improve the adhesion of our bioherbicide [15, 16].

ACKNOWLEDGEMENTS

The authors would like to thank Megan Goers and Julie Byrd for their expert technical assistance.

REFERENCES

1. Chambers, P. A., Lacoul, P., Murphy, K. J. and Thomaz, S. M. 2008, Hydrobiologia, 595, 9-26.
2. Holm, L., Doll, J., Holm, E., Pancho, J. and Herberger, J. 1997, World Weeds: Natural histories and distribution, John Wiley & Sons, Inc, New York, NY, 1129.
3. Richardson, R. J. 2007, Weed Technol., 22, 8-15.
4. Arias, R. S., Netherland, M. D., Scheffler, B. E., Puri, A. and Dayan, F. E. 2005, Pest Manag. Sci., 61, 258-268.
5. Michel, A., Arias, R. S., Scheffler, B. E., Duke, S. O., Netherland, M. and Dayan, F. E. 2004, Mol. Ecol., 13, 3229-3237.
6. Shearer, J. F. 2009, Weed Technol., 24, 202-207.
7. Shearer, J. F. 1998, J. Aquat. Plant Manag., 36, 54-56.
8. Shearer, J. F. and Jackson, M. A. 2006, Biol. Control, 38, 298-306.
9. Owens, D. K. and Wendt, R. C. 1969, J. Appl. Polym. Sci., 13, 1741-1747.

10. Adamson, A. P. and Gast, A. W. 1997, Physical Chemistry of Surfaces, Wiley, New York, 365.
11. Lehr, C. M., Bouwstra, J. A., Bodde, H. E. and Junginger, H. E. 1992, Pharm. Res., 9, 70-75.
12. van Oss, C. J. 1993, Colloids Surf., A, 78, 1-49.
13. van Oss, C. J., Chaudhury, M. K. and Good, R. 1987, J. Adv. Colloid Interface Sci., 28, 35-64.
14. Wu, S. 1970, J. Phys. Chem., 74, 632-638.
15. Esposito, P., Colombo, I. and Lovrecich, M. 1994, Biomaterials, 15, 177-182.
16. Xiang, J. and Li, X. 2006, J. Appl. Polym. Sci., 102, 2608-2615.