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Beach Dune Sand Hydrophobicity Due to the Presence of Beach Vitex (*Vitex rotundifolia* L. f.)

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Conservation and preservation concerns have led to efforts to understand mechanisms of invasiveness and the effects these mechanisms have on the environment. *Vitex rotundifolia* L. f. [beach vitex (BV)] was introduced as a salt-tolerant woody ground cover, but it has since become invasive on primary and secondary dunes in coastal areas of the southeastern United States. Much of its invasive potential may be the result of intense substrate hydrophobicity underneath established stands, which is believed to prohibit seedling establishment by other plants including native plant species. This research was conducted to better understand BV-induced sand hydrophobicity by carrying out dune surveys of BV-infested areas of the South Carolina coast, identifying the compounds responsible for this activity via chemical analysis, and quantifying hydrophobicity persistence by resampling sites following removal of above-ground BV. The findings indicated that sand under BV cover was significantly hydrophobic, that cuticular alkanes from leaves and fruits were responsible for this hydrophobicity, and that extreme substrate hydrophobicity persisted for >3 years following BV removal.

KEYWORDS: Invasive; alkane; cuticle; secondary metabolites

INTRODUCTION

In recent times, environmental concerns have led to the search for increased knowledge about the spread of invasive plants that were introduced as a result of agricultural or horticultural efforts. Researchers have conducted investigations to understand why these plants are invasive to better understand the ecology and interconnected nature of various plant traits, which were not taken into account prior to the introduction of the now-invasive plant. Better understanding of the ecology may also facilitate more efficient management of this invasive plant. This research sought to better understand the cause of beach vitex (BV) (*Vitex rotundifolia* L. f.) invasiveness through an investigation of a previously uncharacterized trait—creation of soil hydrophobicity.

BV was introduced to the Carolina coast of the United States around 1985. It is native to the southwestern Pacific and is found in coastal areas of both islands and continents (*1, 2*). It was transplanted with the hope that its attractive flowers and foliage, salt tolerance, and low-growing habit would allow it to become a widely planted, aesthetically pleasing landscape plant that would also help maintain dune integrity. Unfortunately, this tenaciously spreading shrub has since become a large-scale invasive plant problem. BV dominates primary dune areas and excludes native species (*3*). In places where BV is present in coastal areas of South and North Carolina, it has created large monocultures by shading out native species (*4*). Additional ecological concerns include the belief that BV can impede nesting activities of endangered and federally protected sea turtles (*3*).

Many mechanisms may be responsible for BV invasiveness. These traits include vegetative reproduction, rapid lateral growth, large seed production (*3*), and secondary metabolite production. The extensive, deep (up to 60 cm) root system and lateral growth capabilities are also important characteristics that allow large, intact plants to survive in the hydrophobic substrates they create.

This work focuses on secondary metabolites as a mechanism for helping the plant become invasive. BV fruits and leaves contain many complex metabolites that assist in its survival in the harsh beach dune environment. These compounds include an insect repellant (*5*) and thick layers of cuticular compounds to prevent dehydration. As a result, many studies relating to secondary metabolites of the fruits and their medicinal qualities have been conducted. Compounds discovered include various diterpenes (*6*−*8*) and flavonoids (*9*−*11*). BV has been noted as having thick coatings on leaves and fruits that appear to repel water. In fruits, this coating likely aides in water-based dispersal via ocean currents. This dispersal mechanism is responsible for the plant's pervasiveness throughout the Pacific. BV is present on many volcanic islands, including some that have existed for only a short time (*12*). The absence of a nourishing, fleshy fruit coating suggests that BV is not bird dispersed. Because BV is

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incapable of bird dispersal, it must employ a water-based dispersal mechanism in order to spread from island to island.

Hydrophobic soils have been documented in many areas of the world including beach dunes (*13*), areas subject to frequent fire (*14*), forests (*15*), and desert scrub communities (*16*). Hydrophobic soils were recently identified in the presence of BV (*3*).

The objectives of this study were to (1) characterize multiple sites in South Carolina to confirm the presence of substrate hydrophobicity under multiple stands of BV and the absence of hydrophobicity in areas not infested with BV, (2) understand the variation in hydrophobicity with soil depth, (3) identify the compounds responsible for substrate hydrophobicity, and (4) ascertain the length of time this activity persists in the dune environment following vegetation removal.

MATERIALS AND METHODS

Hydrophobic Soil Field Study. A composite sample of surface (0−5 cm deep) soil was taken from two or more locations under BV cover and under native dune vegetation cover (control sites) at 9 sites in June 2004, 14 sites in June 2005, and 8 sites in 2006. Samples from locations under BV cover were all taken within 10 cm of BV stems. Several sites had only one cover type. BV cover and native cover sites were the same except for the kind of vegetation present. There were no observable differences in topography, proximity to the surf, sun exposure, or substrate characteristics. A single site composite sample served as an experimental unit. The 2004 samples were air-dried, and the time for a 50 g subsample to absorb a drop (200 μL) of water [water drop penetration time (WDPT) (*17*)] was recorded. The 2005 and 2006 samples were air-dried and sieved through a 1.1 mm mesh sieve, and a 30 g subsample was placed in the bottom half of a plastic Petri plate. A drop of distilled water (50 μL) was released from a glass Pasteur pipet held 1 cm above the sand surface. Timing began when the drop fell from the pipet and was stopped when the drop was absorbed into the sand (*18*). Three drops were timed for each Petri plate, and the mean time was calculated for that sample. Timing was stopped at 120 s if the water drops were not absorbed by that time. Timing stopped at 120 s because the authors' observations over the course of 3 h indicated that drops not absorbed by 120 s typically evaporated prior to absorption.

Hydrophobic Tests of Subsurface Soil. During June 2005, soil cores were collected from BV-infested dunes and control dune areas of 13 beachfront sites. Cores were collected using a 1.5 m steel pipe (9.9 cm inner diameter, 1.7 mm wall) with one end sharpened to an inward bevel and with a thick collar welded to the outside of the other end. The pipe was driven 15 cm into the sand before being pivoted to a nearly horizontal orientation and carefully removed from the sand. A specially designed plunger was used to push the collected soil out through the sharpened end into a labeled bag. The pipe was reinserted in the hole and another 15 cm core was extracted and bagged separately. Finally, the pipe was again reinserted and driven (with a sledge hammer hitting a board on top of the steel collar) 30 cm deeper to a depth of 60 cm from the surface, and the 30 cm core was extracted and bagged. Two such cores were taken among the BV, and two cores were taken from the control dunes at each site. The soil was returned to the laboratory, and the root material was carefully removed from each sample. A subsample of the remaining soil was air-dried and sieved through a 1.1 mm mesh sieve before undergoing a test for WDPT as described above.

Chemical Identification of Compounds Causing Sand Hydrophobicity. Both the authors' observations of plant organs and the findings of other researchers with regard to the diverse compounds present in BV suggested that the compounds responsible for sand hydrophobicity would be present on the surfaces of BV leaves and fruits.

An initial extraction solvent selection experiment was conducted at the beginning of the chemical identification phase of the research. Twenty-four glass screw-cap test tubes (25 × 150 mm) with Teflon-lined caps were filled with 100 fruits each. Six of the tubes were extracted with 20 mL of each of the following solvents: acetone, hexane, chloroform, or ethyl ether. The tubes were tumbled for 1 h. Twelve glass Petri plate bottoms were prepared with 25 g of nonhydrophobic beach sand. The extract from two tubes with the same solvent was applied to each Petri plate to give three plates from each solvent extraction method. Solvents were allowed to evaporate under a fume hood overnight. WDPT was determined the following day.

Analysis began with sand extraction and preparation for fractionation. Hydrophobic sand and nonhydrophobic sand (collected from Pawley's Island, SC) in 600 g aliquots were extracted with 600 mL of chloroform each by placing the sand in glass columns and pouring chloroform through the sand matrix. The resulting sand extracts were concentrated to 10 mL using a Buchi rotavapor flash evaporator (Buchi Laboratory Equipment, New Castle, DE) and then further concentrated to 5 mL under a stream of industrial N_2 gas (Airgas Inc., Radnor, PA). BV fruits were surface extracted in chloroform. Four large (25 × 150 mm) glass screw-top test tubes with Teflon caps containing 100 fruits and 20 mL of chloroform each were laid horizontally on an Innova 2100 orbital shaker (90 rpm) for 1 h (New Brunswick Scientific Co., Inc., Edison, NJ). The resulting extract was gravity filtered twice using a Buchner funnel and filter paper (Whatman no. 1). The extract was concentrated to 5 mL under a N_2 gas stream. Similarly, four large screw top test tubes containing 20 fresh green leaves (bisected along the petiole) and 10 mL of chloroform each were tumbled end over end for 1 h. The resulting extract was concentrated to 1 mL under a N_2 gas stream prior to fractionation on TLC plates.

Fruit, leaf, and sand extracts (220 μL) were applied in a line across the absorbent strip at the base of a 5 × 20 cm, 250 μm silica gel plate (Whatman, Clifton, NJ). Hydrophobic bands were located by dripping distilled deionized H_2O down the plate until it encountered the hydrophobic band. A multitude of solvent systems were investigated for separation of hydrophobic compounds from other materials present in the extracts. The mobile phase selected for use in compound separation was an 80:20 methanol/chloroform solvent system that was made in lots of 100 mL and acidified with 50 μL of 1.0 M HCl. The hydrophobic band remained at the plate origin, but other compounds moved from the origin, resulting in good separation. After development, plates were allowed to air-dry under the hood for a period of approximately 20 min before being dried over Drierite (W. A. Hammond Drierite Co., Xenia, OH) for a period of 2 h. The hydrophobic band was then scraped from the plate using a scalpel. The silica was suspended in 5 mL of chloroform in 15 mL conical vials and sonicated for 10 min. The silica was then allowed to settle for 10 min before the sample was filtered through a Pasteur pipet containing glass wool to remove the silica particles. The resulting extract was concentrated to 1 mL under a N_2 gas stream. A glass conical vial containing a 100 μL aliquot of each sample was utilized for GC-MS analysis. A 5 μL sample was injected and analyzed according to the method in the following paragraph.

Samples were run on a (Hewlett-Packard 5890 gas chromatograph with Hewlett-Packard 5971A mass selective detector) GC-MS (Agilent Technologies, Wilmington, DE). The column was a DB-5MS (30 m × 0.25 mm × 0.25 μm film, Agilent Technologies). The carrier flow rate (high-grade helium, 99.997%, Airgas Inc.) was 0.8 mL/min. The initial oven temperature of 60 °C was held for 12 min after injection and then increased at a rate of 12 °C/min to a final temperature of 300 °C, which was held for 12 min, for a total run time of 42.5 min. The split/splitless injector was maintained at 250 °C with a 50:1 split, and the mass transfer line was at 300 °C. The mass selective detector was run in electron impact mode and was turned on at 3.5 min, and fragment masses and abundances between 50 and 500 were tabulated. Two alkane standards (C_5−C_{44}, Supelco; and C_{21}−C_{40}, Fluka) were compared to sample compounds. A combination of retention times from total ion chromatograms and mass spectra were used to establish compound identity. A 1:10 (4 μg/mL) dilution of the C_{21}−C_{40} alkane standard was injected into the GC-MS and used for comparison and quantification.

The alkane standards were also tested for their ability to induce hydrophobicity in sand. The *n*-alkane standard (Supelco, Bellfonte, PA) containing C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{20},

Table 1. Water Drop Penetration Time (WDPT) of Surface (0−5 cm Depth) Sand from South Carolina Beach Dunes Collected in Three Consecutive Years[a]

site	June 2004[b]		June 2005		June 2006	
	BV cover	native cover	BV cover	native cover	BV cover	native cover
Clemmons	0.1	0.1	17.5	0.2	89.0	0.4
Hughes/Perrin			7.7	0.2	120.0	0.3
Irby			114.4	0.2	120.0	0.2
King	0.1	0.1	4.3	0.2	63.8	0.3
Long	0.1	0.1	10.6	0.2	1.3	0.4
Miller			12.2		39.7	
Pop's	120	0.1	1.2	0.2	120.0	0.2
Rainey					120.0	0.4
Richmond	120	0.1	21.7	0.2	64.1	0.2
Robinson			3.8		120.0	
Schultz	120	0.1	120.0	11.3	120.0	
Smith			30.3	0.2	2.7	0.3
Sturgis	120		120.0			
Summer Academy	120	1.0	120.0	0.2		
Williams	0.1	0.1	0.2	0.2		
mean	66.71[c]	0.21	41.70[c]	1.19	81.72[c]	0.30
standard error	21.06	0.11	13.67	1.01	13.47	0.02

[a] Values presented are averages ($n = 3$). [b] Samples tested in 2004 were classified as either <0.10 s or >120 s. [c] Value is significantly different from control cover mean within year, where $\alpha = 0.05$ as determined by an unequal variance *t* test.

C_{24}, C_{26}, C_{30}, C_{36}, C_{40}, and C_{44} was diluted 1:10, 1:100, and 1:1000 in chloroform, and 200 μL aliquots of standard dilutions were applied to 0.25 g of sand in BPI dishes.

Longevity of Hydrophobicity Study. In 2008, all sites were resampled in a manner similar to the sampling conducted in the hydrophobic soil field study experiment. In many cases, this sampling was conducted after the BV had been killed using herbicides and the above-ground portions had been removed. WDPT was determined for each sample as previously noted for samples collected in 2005 and 2006. Locations at sites that had never been under BV cover served as controls for comparison of sites to each other.

Statistical Analysis. Statistical comparisons for activity characterization and longevity listed at the ends of **Tables 1** and **4** were calculated using an unequal variance *t* test. This test was selected because the variance of the native cover site WDPTs was different from the variance of the BV cover site WDPTs. Additionally, this test is more robust than Student's *t* test or an ANOVA, allowing for statistical validity despite the fact that data set variation assumptions are skewed slightly by the large number of 0.1 and 120 s data values. Statistical comparisons for tests of subsurface soil listed at the end of **Table 2** were conducted using ANOVA and linear contrasts. Statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Hydrophobic Soil Field Study. Five of the nine sites sampled under BV cover in 2004 demonstrated extreme hydrophobicity (WDPT > 120 s), whereas the other four exhibited no hydrophobicity (WDPT < 1 s) (**Table 1**; **Figure 1**). Only one of the nine samples taken in 2004 from control dunes without BV was weakly hydrophobic (WDPT = 1−30 s). In the more carefully measured 2005 and 2006 samples, all but one of the BV cover samples showed some level of hydrophobicity: 9 of 26 samples were extremely hydrophobic, 6 samples were moderately hydrophobic (WDPT = 30−120 s), and 10 samples were weakly hydrophobic. All but one of the control dune samples was not hydrophobic: 19 of 20 samples were not hydrophobic, with 1 sample being weakly hydrophobic. In all cases for the more carefully measured data (2005 and 2006), the hydrophobicity was much stronger in BV-infested areas than in nearby control areas (i.e., in the only hydrophobic control dune site, the WDPT was 11.3 s, whereas the corresponding BV-covered dune at the same location had a WDPT of >120 s).

Table 2. Water Drop Penetration Time (WDPT) of Beach Sand from South Carolina Beach Dunes at Three Depth Ranges (0−15, 15−30, and 30−60 cm) at 13 Sites[a]

site	BV cover			native cover		
	0−15 cm	15−30 cm	30−60 cm	0−15 cm	15−30 cm	30−60 cm
Clemmons	76.04	37.36	0.37	0.15	0.14	0.15
Hughes	4.90	1.14	0.14			
Irby	4.25	0.21	0.15	0.17	0.17	0.16
King	0.20	0.16	0.25	0.17	0.16	0.14
Long	0.15	0.19	0.15	0.16	0.17	0.16
Miller	0.20	0.17	0.16			
Perrin				0.16	0.18	0.18
Robinson	1.88	0.24	0.23			
Schultz	120	15.27	0.17	4.23	0.71	0.19
Smith	1.68	0.16	0.15	0.16	0.15	0.16
Sturgis	45.15	0.17	0.16	0.67	0.17	0.16
Summer Academy	62.66	18.93	0.16	0.21	0.17	0.18
Williams	0.16	0.17	0.15	0.15	0.17	0.14
mean	26.44[b]	6.18	0.19	0.62	0.22	0.16
standard error	11.61	3.41	0.02	0.40	0.05	0.01

[a] Values presented are averages ($n = 6$). [b] Value is significantly different from all other depth × cover means, where $\alpha = 0.05$ as determined by ANOVA.

Figure 1. Water droplets placed on hydrophobic (left) and nonhydrophobic (right) sands. Note rounded drop on hydrophobic sand compared to completely absorbed water on nonhydrophobic sand [video showing water droplet on nonhydrophobic sand followed by hydrophobic sand located at http://www.northinlet.sc.edu/resource/vitex_files/aug05/Hydro1.wmv (Charles A. Gresham)].

BV had been present on most of the sites that were sampled since 1990 or 1991. It was widely planted as an erosion preventative in the aftermath of Hurricane Hugo (landfall in September 1989). As a result, it had been present at most of the sites for approximately 15 years, and its presence resulted in substrate hydrophobicity that is both intense and pervasive.

Chemical creation of substrate hydrophobicity would be an effective defensive mechanism against competition. Seedling establishment would be prohibited because the upper soil layers would not contain water in sufficient quantities to allow seedlings to survive. Running vegetatively reproducing plants (such as BV) with large root systems would be favored in such conditions. Adams et al. (*16*) reported a similar phenomenon for desert shrub vegetation in southern California. Hydrophobic layers were found in soils beneath hummocks of desert vegetation but not in soils where hummocks were absent.

Hydrophobic Tests of Subsurface Soil. The results of the WDPT tests are summarized in **Table 2**. Only 1 of 10 control (native cover) sites demonstrated weak hydrophobicity in the surface layer. None of the control sites were hydrophobic at a

depth of 15−30 or 30−60 cm. With regard to surface layer hydrophobicity at the BV sites, 1 of the 12 sites was extremely hydrophobic, 3 sites were moderately hydrophobic, 4 sites demonstrated weak hydrophobicity, and 4 sites were not hydrophobic. The hydrophobicity decreased with depth at the hydrophobic locations. In fact, only 4 of the BV sites were weakly hydrophobic at the 15−30 cm depth layer, and none of the sites were hydrophobic 30−60 cm below the dune surface. An ANOVA indicated that there was a highly significant cover effect (mean WDPT for BV cover over all depths and sites was 14.24 s, whereas the mean WDPT was 0.35 s for control soil at all depths and sites). A significant depth effect was also noted in the BV cover areas as part of the significant depth by cover interaction. The mean WDPT for the 0−15 cm depth was 26.44 s, whereas the mean WDPT values for the 15−30 and 30−60 cm depths were 6.18 and 0.19 s, respectively. The mean WDPT for the 0−15 cm depth under BV was much lower than the WDPTs of surface soil samples (0−5 cm, **Table 1**) at the same sites. This indicates that the compounds responsible for the hydrophobic response were present in the highest concentrations in the first few centimeters of the soil surface. Some hydrophobic effect was seen at deeper depths, but the effect was less intense.

Chemical Identification of Compounds Causing Substrate Hydrophobicity. Chloroform was found to be the best solvent for the extraction of compounds causing hydrophobicity. In all tests, chloroform extracts of BV fruits caused nonhydrophobic sands to hold drops of water for an excess of 120 s, whereas nonhydrophobic sands treated with hexane, acetone, and ethyl ether extracts held drops of water for averages of 2.9 s (standard deviation = 1.7), 15.0 s (standard deviation = 28.7), and 23.5 s (standard deviation = 22.3), respectively. As a result, chloroform was selected as the extraction solvent for use in the remainder of the extraction and purification studies.

Initial GC-MS examination of complete extracts from hydrophobic and nonhydrophobic sands demonstrated complex metabolite profiles in the hydrophobic sand that contrasted significantly with the small number of compounds detected in nonhydrophobic sand. Fractionation was required to identify compounds responsible for substrate hydrophobicity.

Extracts of fruits were found to produce areas of hydrophobicity on silica TLC plates. TLC separation and hydrophobic band detection method development was employed so that simplified total ion chromatograms with fewer peaks could be obtained. Hydrophobic sand extracts, fruit extracts, and leaf extracts behaved similarly on TLC plates in response to various mobile phases with regard to how the hydrophobic bands migrated. Movement and solubility of compounds causing hydrophobicity matched solubilities of compounds found in cuticular alkanes and lipids of plants previously examined (*19*). Due to difficulty moving the hydrophobic compounds from the origin without dispersing them widely across the plate, a solvent system was selected that retained the hydrophobic band at the origin while moving other compounds that were seen visually and through exposure of plates to short- and long-wave UV light ranges away from the origin.

GC-MS total ion chromatograms of fractionated extracts (**Figure 2**) indicated that GC retention time and MS fragmentation patterns of compounds found in TLC-fractionated extracts of BV leaves and fruits matched chromatograms and spectra of compounds from similarly prepared hydrophobic sand extracts. The compounds of interest were not found in nonhydrophobic sand extracts or solvent controls. MS library comparison identified peaks of interest as alkanes. This was confirmed through inspection of MS data and comparison with alkane standards. Comparisons were conducted using the base peak, the mass ion, and eight additional major fragments based on fragment size and frequency in relation to the base peak. Alkane standards produced MS spectra that were nearly identical to the spectra of the peaks of interest. All samples had base peaks at 57. Mass ions and percentages of base peaks are presented in the following paragraphs in brackets and parentheses.

The two lightest alkanes in the $C_{21}-C_{44}$ alkane standard were observed only in the standard: C_{21} [296 (0.9)] and C_{22} [310 (0.7)]. The next six alkanes ($C_{23}-C_{28}$) were identified in the alkane standard and in hydrophobic sand only and were C_{23}−standard [324 (0.7)], hydrophobic sand [324 (0.4)]; C_{24}−standard [338 (0.8)], hydrophobic sand [338 (0.7)]; C_{25}−standard [352 (0.8)], hydrophobic sand [352 (0.9)]; C_{26}−standard [366 (0.8)], hydrophobic sand [366 (0.8)]; C_{27}−standard [380 (0.7)], hydrophobic sand [380 (0.7)]; and C_{28}−standard [394 (0.7)], hydrophobic sand [394 (0.8)]. The next nine alkanes ($C_{29}-C_{37}$) were identified in the alkane standard, hydrophobic sand, fruit extract, and leaf extract and were C_{29}−standard [408 (0.6)], hydrophobic sand [408 (0.6)], fruit extract [408 (0.7)], leaf extract [408 (0.7)]; C_{30}−standard [422 (0.5)], hydrophobic sand [422 (0.7)], fruit extract [422 (0.5)], leaf extract [422 (0.4)]; C_{31}−standard [436 (0.5)], hydrophobic sand [436 (0.6)], fruit extract [436 (0.2)], leaf extract [436 (0.2)]; C_{32}−standard [450 (0.5)], hydrophobic sand [450 (0.5)], fruit extract [450 (0.6)], leaf extract [450 (0.7)]; C_{33}−standard [464 (0.4)], hydrophobic sand [464 (0.6)], fruit extract [464 (0.1)], leaf extract [464 (n.d.)]; C_{34}−standard [478 (0.3)], hydrophobic sand [478 (0.5)], fruit extract [478 (0.5)], leaf extract [478 (0.6)]; C_{35}−standard [492 (0.4)], hydrophobic sand [492 (0.5)], fruit extract [492 (0.5)], leaf extract [(0.02)]; C_{36}−standard [506 (nd)], hydrophobic sand [506 (nd)], fruit extract [506 (nd)], leaf extract [506 (nd)]; and C_{37}−standard [520 (nd)], hydrophobic sand [520 (nd)], fruit extract [520 (nd)], leaf extract [520 (nd)].

A summary of alkanes found in hydrophobic sand, fruit, and leaf samples can be found in **Table 3**. Following identification of compounds, the alkane concentrations were determined for the nonhydrophobic and hydrophobic sand as well as the fruit and leaf extracts. Leaves contained more cuticular alkanes per gram of tissue than fruits (>10 times more in some cases). This may be partially explained by the fact that leaves have a much greater surface area to mass ratio than fruits.

In the hydrophobic sand, the average content of the alkanes numbered $C_{23}-C_{37}$ was 0.477 μg/g of sand. Fruits produced an average of 0.212 μg of each alkane numbered $C_{29}-C_{37}$ per gram of fresh weight, whereas leaves produced an average of 4.907 μg of each alkane numbered $C_{29}-C_{37}$ per gram of fresh weight. When calculations were conducted on a per fruit or per leaf basis, average production of alkanes $C_{29}-C_{37}$ was 4.424 μg/fruit and 12.116 μg/leaf. Each fruit produces enough alkanes to make more than 10 g of sand hydrophobic, whereas each leaf produces enough alkanes to make >30 g of sand hydrophobic. It is unlikely that this level of efficacy would be achieved in nature because alkane transfer would occur through physical interaction of fruit, leaf, and sand surfaces and not through solvent extraction, which is a more efficient process.

Some alkanes ($C_{23}-C_{28}$) were found in the hydrophobic sand but not in the fruit or leaf extract samples. These compounds are likely degradation products of the other longer *n*-alkane chains that are produced on the surfaces of BV leaves and fruits. Beach sands are subjected to massive amounts of heat from the sun. Other researchers have noted that high temperatures

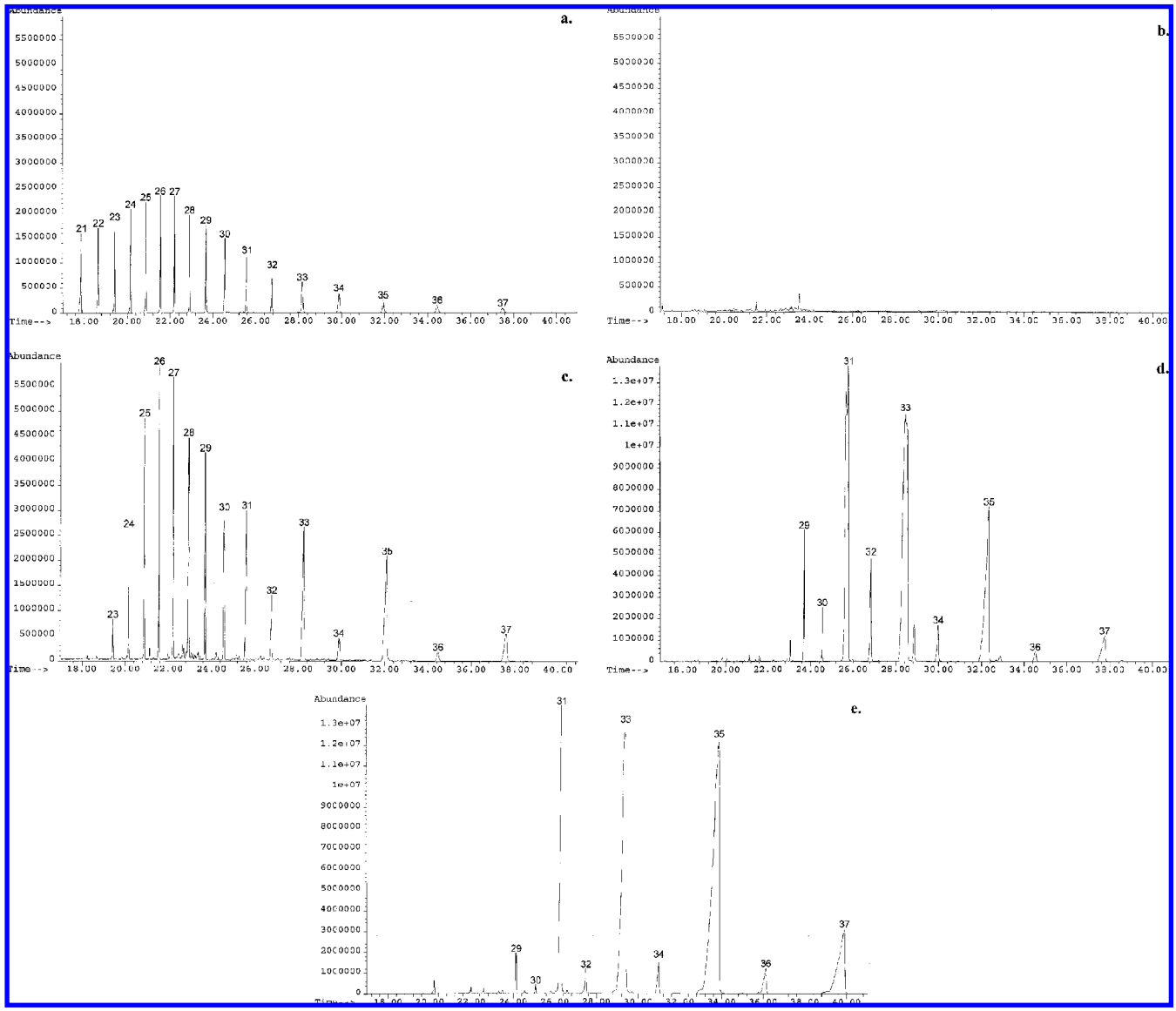

Figure 2. Total ion chromatograms of alkane standard (**a**), nonhydrophobic sand (**b**), hydrophobic sand (**c**), BV fruits (**d**), and BV leaves (**e**). Numbers above peaks indicate number of carbons in the *n*-alkane that corresponds to the peak. Peaks with no number were not identified as alkanes through observation of MS data. Asymmetric peaks are visible for BV fruit and leaf chromatograms due to the large concentrations of alkanes in these two samples. *Y*-axis scales for fruit and leaf chromatograms are larger due to much higher levels of alkanes present in these samples.

can cause a reduction in chain length of alkane chains, resulting in an increase in shorter alkane soil content (*20*). Biological degradation is unlikely to have been responsible for the presence of shorter alkanes because most microbes do not break down alkanes of this length, and major breakdown products would be fatty acids (*21*). Because no alkanes were found in the nonhydrophobic sand samples, the alkanes identified in the hydrophobic sand are the result of BV presence. Stems and roots were not investigated in this experiment. Stems were not investigated because they are responsible for a small portion of the total plant material surface area on the dune. Roots were not investigated because the majority of the hydrophobicity was found in the surface layer of the sand (a layer that contains few roots). Additionally, alkanes C_{31}, C_{33}, C_{35}, and C_{37} were the most abundant alkanes in hydrophobic sand under BV vegetation. These four compounds were also the most prevalent in the leaves and fruits.

As a confirmation that the compounds identified in the GC-MS total ion chromatogram were indeed those responsible for sand hydrophobicity, extracts were added to nonhydrophobic sand and evaporated to dryness. Fractionated hydrophobic sand extracts, leaf extracts, and fruit extracts were shown to induce significant hydrophobicity in nonhydrophobic sand. A 200 μL aliquot of a solution containing 0.4 μg/mL each of $C_{21}-C_{40}$ caused weak hydrophobicity in 0.25 g of previously nonhydrophobic sand. This equates to 0.32 μg of each alkane/g of sand as opposed to an average of 0.477 μg/g present in natural hydrophobic sand. An alkane standard (Supelco C_5-C_{40}) was also used to successfully create hydrophobic sand in the laboratory setting. The 1:10 dilution (Supelco C_5-C_{40}) (880 μg of alkane/g of sand) failed to evaporate and dry on the sand, whereas higher dilutions did evaporate, allowing WDPT determination. Sand treated with a 1:100 dilution (88 μg of alkane/g of sand) had a WDPT of 3 min, whereas sand treated with a 1:1000 dilution (8.8 μg of alkane/g of sand) had a WDPT of approximately 10 s. Control sand held a drop for <1 s. Because two-thirds of the alkanes contained in the standard were shorter chains (chains that would cause less substrate hydrophobicity), these values support the total alkane values from **Table 3**. Field-

Table 3. Quantification and Identification of Alkanes in Various BV-Associated Extracts[a]

alkane		estimated alkane content[b]			
carbon no.	RT[c] (approx)	hydrophobic (μg/g)	nonhydrophobic (μg/g)	fruits (μg/g)	leaves (μg/g)
21	17.8	nd[d]	nd	nd	nd
22	18.6	nd	nd	nd	nd
23	19.4	0.057	nd	nd	nd
24	20.1	0.172	nd	nd	nd
25	20.8	0.304	nd	nd	nd
26	21.5	0.348	nd	nd	nd
27	22.2	0.380	nd	nd	nd
28	22.9	0.358	nd	nd	nd
29	23.6	0.352	nd	0.028	0.123
30	24.5	0.309	nd	0.015	0.030
31	25.6	0.423	nd	0.382	2.225
32	26.7	0.232	nd	0.057	0.180
33	28.3	0.825	nd	0.649	7.148
34	29.8	0.184	nd	0.043	0.519
35	31.8	1.775	nd	0.524	22.545
36	34.4	0.194	nd	0.028	1.205
37	37.5	1.240	nd	0.185	10.188
total		7.153	nd	1.911	44.163

[a] There was no correction for percent recovery. [b] Limits of detection: 0.2 μg/mL (GC-MS sample), 7.576 ng/g sand, 8.636 ng/fruit, 11.364 ng/leaf, 0.415 ng/g fruit, 0.545 ng/g leaf. [c] Retention time of GC column. [d] nd, none detected.

collected hydrophobic sand (WDPT > 120 s) contained 7.153 μg of alkane/g of sand.

The results show that the compounds responsible for sand hydrophobicity near areas infested with BV are long-chain alkanes ranging from C_{23} to C_{37}. These results are supported by those of Horn and McIntosh (*22*), who found that alkanes were responsible for causing beach sand hydrophobicity in New Zealand; however, they did not link this hydrophobicity to the presence of specific plants. These compounds are produced by BV and accumulate in the cuticles of BV fruits and leaves. As leaves and fruits drop into the substrate, these surface wax components are deposited in the soil matrix. This deposition is likely aided by heating as a result of direct solar exposure and physical contact with sand particles. Over time, a buildup of these compounds in the substrate yields the hydrophobicity observed in the presence of BV. Substrate hydrophobicity likely plays an important ecological role as it helps maintain BV monocultures by preventing seedling establishment and slowing ecosystem recovery following removal of this highly invasive exotic shrub.

Longevity of Hydrophobicity Study. Resampling and analysis of sites in 2008 (**Table 4**) demonstrated that the sand hydrophobicity persisted in the substrate long after the removal of BV from the area. Results at some sites showed that areas where BV had been absent for two to three seasons maintained extremely hydrophobic substrates. The fact that some sites exhibited lower hydrophobicity than others is likely the result of variation in the amount of vegetation that covered each individual site prior to vegetation removal.

These findings indicate that substrate hydrophobicity induced by BV has a negative impact on the fragile dune environment that persists long after this exotic invasive plant has been eradicated (more than three seasons following vegetation removal). As a result, plants that are more able to survive in this hydrophobic substrate should be considered for planting as part of site revegetation efforts. The introduction of container-grown plants as an alternative to broadcasting seeds might yield greater success. Dune hydrophobicity would be more likely to negatively affect small seedlings than larger plants. We are currently investigating methods for remediation of substrate hydrophobicity so that dune community recovery might be accelerated.

Table 4. Water Drop Penetration Time (WDPT) for Site Resampling Conducted in June 2008[a]

site	BV cover	native cover (control)	vegetation removal date
Clemmons		0.1	June 2005
Hughes	120		Sep 2005
Irby	108	0.1	March 2007
Long	2.0	0.2	June 2007
Miller	120		still vegetated
Pop's	120	0.3	April 2007
Rainey	120		June 2006
Richmond	1.0	0.1	Nov 2005
Robinson	3.1		aug 2007
Smith	58.8	0.1	March 2007
Sturgis	5.2		still vegetated
Summer Academy	120		March 2007
mean	70.80[b]	0.15	
standard error	17.06	0.03	

[a] BV cover includes areas remaining under BV cover and also areas where BV cover had been removed. Native cover areas never had BV cover. [b] Value is significantly different from native cover control mean, where $\alpha = 0.05$ as determined by an unequal variance *t* test.

ABBREVIATIONS USED

BV, beach vitex (*Vitex rotundifolia*); GC, gas chromatograph; MS, mass spectrometer; TLC, thin layer chromatography; WDPT, water drop penetration time; WDPT > 120 s, extremely hydrophobic; WDPT = 30−119 s, moderately hydrophobic; WDPT = 1−30 s, weakly hydrophobic; WDPT < 1 s, not hydrophobic.

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