Induction of Haustorium Development by Sphaeropsidones in Radicles of the Parasitic Weeds Striga and Orobanche. A Structure-Activity Relationship Study

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Induction of Haustorium Development by Sphaeropsidones in Radicles of the Parasitic Weeds *Striga* and *Orobanche*. A Structure–Activity Relationship Study

Mónica Fernández-Aparicio,[†] Marco Masi,[‡] Lucia Maddau,[§] Alessio Cimmino,[‡] Marco Evidente,[‡] Diego Rubiales,[∥] and Antonio Evidente*[,‡]

[†]Agroécologie, INRA UMR1347, BP 86510, F-21000 Dijon, France

[‡]Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, Via Cintia 4, 80126, Napoli, Italy

[§]Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100, Sassari, Italy

[∥]Institute for Sustainable Agriculture, CSIC, Apdo. 4084, 14080 Córdoba, Spain

ABSTRACT: Crop attack by parasitic weeds such as *Striga* and *Orobanche* occurs through developmental processes triggered by host chemodetection. Seeds of those weed species remain dormant in the soil until germination is triggered by host root exudates. The development of haustorium, a parasitic plant organ that invades the host to withdraw its nutrients, is also initiated in Orobanchaceae by host molecular cues. The induction of haustorium development by exogenous signals has previously been reported for *Striga* but not for *Orobanche* species. In this work, we demonstrate that sphaeropsidone and *epi*-sphaeropsidone, two phytotoxic cyclohexene oxides isolated from the fungus *Diplodia cupressi*, a causal agent of cypress canker, induce haustorium development in radicles of the parasitic weeds *Striga hermonthica*, *Orobanche crenata*, and *Orobanche cumana*. This is the first report of chemical stimulation of haustorium development in radicles of *Orobanche* in the absence of host. In addition, SAR studies were carried out by testing the haustorium-inducing activity of the natural cyclohexene oxides, seven already known and four new hemisynthetic derivatives, in *O. cumana*, *O. crenata*, and *S. hermonthica*, to find a molecular specificity model required for haustorium induction. The results suggested that the haustorium-inducing activity is due to the possibility to convert the natural sphaeropsidone and natural and hemisynthetic derivatives in the corresponding 4-methoxyquinone and that the stereochemistry at C-5 also seems to affect this activity.

KEYWORDS: *Striga hermonthica, Orobanche crenata, Orobanche cumana, cyclohexene oxides, attachment organ, haustorium-inducing factor*

INTRODUCTION

Root parasitic plants have evolved to exploit another plant root system to fulfill their water and nutrient requirements. Two main types of parasitic plants can be recognized regarding their photosynthetic status: hemiparasitic plants perform some degree of photosynthesis but are dependent on the host mainly for water and inorganic nutrients, while holoparasitic plants lack of photosynthetic machinery and in the nutrient diversion from the host they include all their required reduced carbon. Hemiparasitic plants are also classified in obligated or facultative parasites depending on their ability to develop an autotrophic way of life in the absence of a suitable host.[1] Plants from up to 28 dicotyledonous families use the plant-parasitic strategy to obtain competitive advantage from neighboring host plants.[2] In all of them, the core feature of their parasitic strategy is a specialized host-invasive organ called haustorium that develops sequential functions of host attachment, penetration, and connection with host vascular tissues.[3,4] At least in one family of parasitic plants, the Orobanchaceae, the haustorium development is initiated by host-derived metabolites.[5] Some members of this family, i.e., the hemiparasitic plants *Striga* spp. and the holoparasitic plants *Orobanche* spp., are weeds to which despite their high negative economic impact in agriculture there is not effective and or practicable control and thus the development of innovative control strategies is urgent.

In vitro screenings of host-derived factors that initiate developmental processes in parasitic plants are essential in breeding programs for parasitic plant resistance.[6] *Striga* haustorium can be monitored in vitro by exposing *Striga* seedlings to inducing factors. Upon detection of haustorium-inducing factors, the *Striga* seedling initiates haustorium development and undergoes quick morphological changes that prepare it for its first step in the host invasion process: attachment to host root. Long haustorial hairs with functions of adhesion and attachment develop from elongation of epidermal cells surrounding the haustorium apex.[4] Despite the fact that *Striga* and *Orobanche* haustoria have a common evolutionary origin,[7] fundamental differences exist at their host-preattached haustorial stage. Morphologically, *Orobanche* haustorium is by far less defined. It lacks haustorial hairs but rather it develops

Received: April 27, 2016
Revised: May 23, 2016
Accepted: June 6, 2016

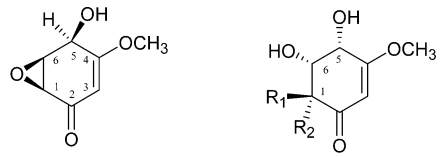
DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

short secretory papillae with function of the adhesion surface.[8] Another fundamental difference with *Striga* is that in *Orobanche* species, the chemistry of haustorium initiation is completely unknown. Several authors have suggested that *Orobanche* species do not require host chemical factors for haustorium development, which would make *Orobanche* the notable exception in the Orobanchaceae.[8−10] In consequence, in vitro assays of haustorium-inducing factors or haustorium-inducing root exudates have been unfeasible in *Orobanche*, hampering the screening for low-inducer genotypes in crop germplasm collections.

Several phenols, flavonoids, and quinones have been identified as factors inducing both terminal and lateral haustoria in obligated hemiparasitic plants, i.e., *Striga hermonthica* and facultative hemiparasitic plants, i.e., *Triphysaria versicolor*.[4,13−15] The Atsatt's hypothesis[16] that parasitic plants evolved to use defense metabolites as host recognition signals is supported by the fact that many haustorium-inducing factors are structurally similar to phytotoxins produced by allelopathic plants. There is a similar gene expression in parasitic roots when they are exposed to host contact or allelochemicals.[5,16] The haustorium development in hemiparasitic Orobanchaceae is under redox control mediated by cycling between the reduced and oxidized states of the haustorial-inducing compounds.[17] Similar models in the quinone-mediated actions of both phytotoxic allelopathy and haustorium initiation are proposed.[5,10,18]

Some fungal metabolites are able to interfere with weed parasitism either by phytotoxic action on germination or growth inhibition or by triggering developmental programs in a suicidal fashion, i.e., in the absence of a host. These new compounds constitute alternative candidate ingredients for the development of new herbicides. Parasitic weeds that can be targeted include species of high economic important members of *Orobanche*, *Phelipanche*, and *Striga* genera.[11,12,19−22]

Sphaeropsidone and *epi*-sphaeropsidone (**1** and **2**, Figure 1) are two cyclohexene oxides isolated together, several



1 R_1=H, R_2=OH

2 R_1=OH, R_2=H

3 R_1=Cl, R_2=H

4 R_1=H, R_2=Cl

Figure 1. Structures of sphaeropsidone and *epi*-sphaeropsidone (**1** and **2**) and chlorosphaeropsidone and epichlorosphaeropsidone (**3** and **4**).

sphaeropsidins[23] as the main phytotoxins produced from *Diplodia cupressi*, the causal agent of cypress (*Cupressus sempervirens* L.) canker in the Mediterranean basin.[24] Successively, two chlorinated cyclohexenenones, closely related to **1** and **2** and named chlorosphaeropsidone and epichloropsphaeropsidone (**3** and **4**, Figure 1), were isolated from the same fungal culture filtrates.[25] They have phytotoxic activity causing necrosis and wilting in *Quercus* species and in tomato and antifungal activity by inhibiting mycelial growth in species of *Phytophthora*.[26]

In a previous study, among fungal and plant phytotoxins, sphaeropsidones were identified as metabolites able to induce morphological changes in the *Orobanche* radicle that resemble the attachment organ.[11] Because of the nuanced morphology of *Orobanche* preattached haustorium and to the fact that no chemicals with haustorium-inducing activity have been previously identified for any *Orobanche* species, we have included in the present work *S. hermonthica* as a living marker for haustorium induction due to its very distinct haustorium morphology. The present article confirms the haustorium-inducing nature of sphaeropsidone activity and that it acts across the parasitic weed genera *Orobanche* and *Striga*. Haustorium-inducing assays of sphaeropsidones were performed in *O. cumana*, *O. crenata*, and *S. hermonthica*. In addition, structure−activity relationships were carried out by assaying two natural analogues (**3** and **4**), seven already known and four new sphaeropsidones derivatives[26] prepared by chemical transformation of **1** and **2**.

MATERIALS AND METHODS

General Experimental Procedures. IR spectra were recorded as deposit glass film on a 5700 FT-IR spectrometer (Thermo Electron Corp. Nicolet, Madison, WI, USA), and UV spectra were measured in MeCN on a V-530 spectrophotometer (Jasco, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded at 400/100 or 500/125 MHz in $CDCl_3$ on Bruker spectrometers (Karlsruhe, Germany). The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra.[27] ESI spectra were recorded on a 6230 TOF LC/MS instrument (Agilent Technologies, Milan, Italy). Analytical and preparative TLC were performed on silica gel (Kieselgel 60, F_{254}, 0.25 and 0.5 mm, respectively) plates (Merck, Darmstadt, Germany). The spots were visualized by exposure to UV radiation (253) or by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. Column chromatography was performed using silica gel (Kieselgel 60, 0.063−0.200 mm) (Merck).

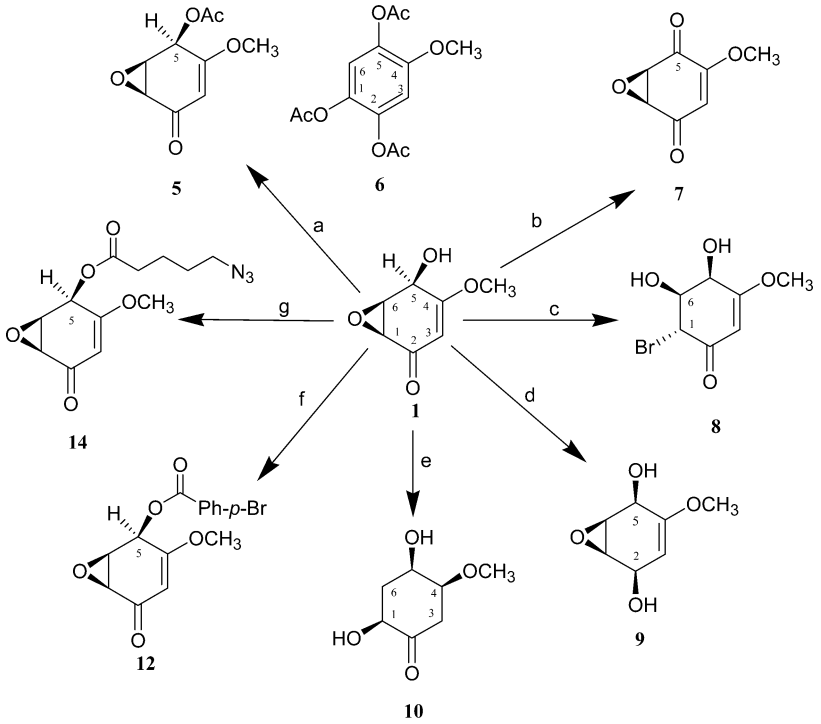
Fungal Strains. The *D. cupressi* strain used in this study was purchased from Centraalbureau voor Schimmelcultures of Baarn (Netherland). Pure culture was maintained on potato dextrose agar (PDA) (Fluka, Sigma-Aldrich Chemic GmbH, Buchs, Switzerland) and stored at 4 °C in the collection of Dipartimento di Agraria, Università di Sassari, Italy, as 261.85 CBS. The fungus was grown as previously reported[26]

Extraction and Isolation of Sphaeropsidones. One-week-old colonies of the fungus on PDA in 9 cm Petri dish were cut off and blended for 30 s with 100 mL of sterile distilled water to prepare the inoculum for liquid fermentation. Each 2 L Erlenmeyer flask containing 400 mL of modified Czapek medium supplemented with 2% corn meal (pH 5.7) was seeded with 5 mL of the mycelial suspension and then incubated at 25 °C for 4 weeks in darkness. The culture filtrates (15 L) were acidified and extracted exhaustively with EtOAc as previously reported.[24] The organic extract, obtained as a brown−red oil (9.2 g), was chromatographed on a silica gel column (110 cm × 5 cm) eluted with 3 L of $CHCl_3$/*i*-PrOH (9:1, v/v), affording nine groups of homogeneous fractions. The residues (3.6 g) of fractions 4−7 were combined and further purified by a silica gel column (90 cm × 4 cm) eluted with 2.5 L of $CHCl_3$/*i*-PrOH (9:1, v/v), yielding six groups of homogeneous fractions. The residue of fraction 3 was crystallized from EtOAc/*n*-hexane (1:5, v/v), yielding sphaeropsidone, **1** (R_f 0.40, 2.3 g, 153.3 mg/L) (Figure 1), as white needles. The mother liquors were further purified by column chromatography on silica gel (75 cm × 3 cm) eluted with 1 L of petroleum ether−Me_2CO (7:3, v/v), affording *epi*-sphaeropsidone, **2** (R_f 0.53, 725 mg, 48.3 mg/L) (Figure 1), as a homogeneous oil. Chlorosphaeropsidone, **3**, and epichlorosphaeropsidone, **4** (Figure 1), were purified from the same culture filtrate, and the purification procedures for these compounds have been described previously.[25]

Preparation of Sphaeropsidones Derivatives (5−11). The 5-*O*-acetyl, **5**, 2,4,5-triacetylanisole, **6**, 1,4-dione, **7**, bromohydrin, **8**, 1,4-diol, **9**, and 2,4-dihydroxycyclohexanone, **10**, derivatives of sphaeropsidone, **1** (Scheme 1) as well as the 5-*O*-acetylderivative, **11**, of *epi*-

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

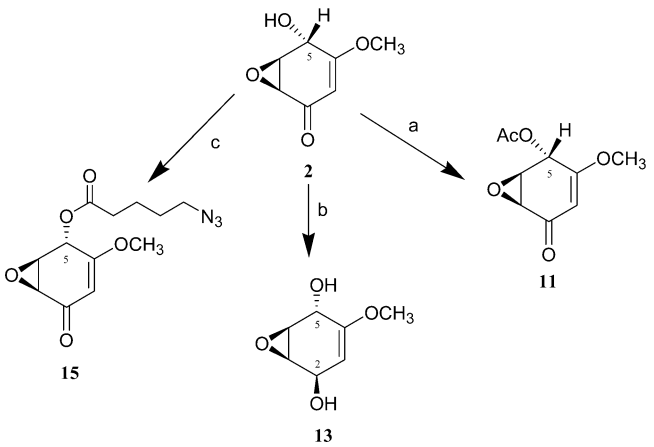
Scheme 1. Synthesis of Sphaeropsidone Derivatives (5−10, 12, and 14)[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, 80 °C; (b) MnO_2, CH_2Cl_2, rt; (c) Li_2NiBr_4, THF, rt; (d) $NaBH_4$, MeOH, rt; (e) H_2, Pd 10%, MeOH, rt; (f) CH_3CN, DMAP, BrC_6H_4COCl, rt; (g) CH_2Cl_2, pyridine, DCC, $C_5H_9N_3O_2$, 0 °C 1 h and rt 2 h.

s2 sphaeropsidone, **2** (Scheme 2), were prepared according to the procedures previously reported.[26]

Scheme 2. Synthesis of *epi*-Sphaeropsidone Derivatives (11, 13, and 15)[a]



[a]Reagents and conditions: (a) Ac_2O, AcONa, 80 °C; (b) $NaBH_4$, MeOH, rt; (c) CH_2Cl_2, pyridine, DCC, $C_5H_9N_3O_2$, 0 °C 1 h and rt 2 h..

5-*O*-*p*-Bromobenzoyl Ester of Sphaeropsidone (12). Sphaeropsidone, **1** (5 mg), was dissolved in CH_3CN (0.5 mL), and DMAP (4-dimethylaminopyridine) (10 mg) and *p*-bromobenzoyl chloride (10 mg) were added. The reaction mixture was stirred at room temperature for 4 h and then evaporated under reduced pressure. The residue (10.0 mg) was purified by TLC on silica gel eluted with $CHCl_3$/*i*-PrOH (95:5, v/v), giving derivatives **12** (3.65 mg, R_f 0.78) (Scheme 1, as uncolored oil. **12** had: UV λ_{max} (log ε) 250 (4.07) nm. IR ν_{max} 1728, 1670, 1618, 1590 cm^{-1}. ^1H NMR, δ: 8.01 (d, J = 8.7 Hz, H-2′ and H-6′), 7.63 (d, J = 8.7 Hz, H-3′ and H-5′), 6.19 (d, J = 2.5 Hz, H-5), 5.35 (br s, H-3), 3.87 (dd, J = 4.3 and 2.5, H-6), 3.71 (s, OMe), 3.54 (dd, J = 4.3 and 1.7, H-1). ESIMS (+) m/z 364 $[M + 2 + Na]^+$, 362 $[M + Na]^+$, 342 $[M + 2 + H]^+$, 340 $[M + H]^+$.

5-*O*-5′-Azidopentanoyl Ester of Sphaeropsidone (14). To compound **1** (5.0 mg) dissolved in anhydrous CH_2Cl_2 (4.5 mL) and pyridine (100 mL) were added DCC (*N*,*N*′-dicyclohexylcarbodiimide) (5 mg) and 5-azidopentanoic acid (20 μL). The reaction was left at 0 °C for 1 h and then at room temperature for 2 h. The reaction was stopped by evaporation under N_2. The residue (10 mg) was purified by TLC on silica gel eluted with $CHCl_3$/*i*-PrOH (95:5, v/v), giving derivatives **14** (7.90 mg, R_f 0.74) (Scheme 1), as uncolored oil. **14** had: UV λ_{max} (log ε) 253 (3.51) nm. IR ν_{max} 2100, 1746, 1672, 1618 cm^{-1}. ^1H NMR, 5.97 (d, J = 2.9 Hz, H-5), 5.30 (br s, H-3), 3.76 (dd, J = 4.0 and 2.9, H-6), 3.70 (s, OMe), 3.50 (dd, J = 4.0 and 1.4, H-1), 3.32 (t, J = 6.5 Hz, CH_2-5′), 2.53 (t, J = 7.3 Hz, CH_2-2′), 1.83−1.76 (m, CH_2-3′), 1.71−1.64 (m, CH_2-4′). ESIMS (+) m/z 304 $[M + Na]^+$, 282 $[M + H]^+$.

$NaBH_4$ Reduction of *epi*-Sphaeropsidone (13). To *epi*-Sphaeropsidone, **2** (5 mg), dissolved in MeOH (15 mL), was added $NaBH_4$ (5 mg) under stirring at room temperature for 30 min. The mixture was neutralized with 0.1 M HCl, extracted with CH_2Cl_2 (3 × 30 mL) and dried (Na_2SO_4). The oily residue was purified by preparative TLC, using $CHCl_3$−*i*-PrOH (9:1) for elution to give the derivative **13** as white needles (3.45 mg, R_f 0.37) (Scheme 2). **13** had: UV λ_{max} (log ε) < 220 nm. IR ν_{max} 3413, 1671 cm^{-1}. ^1H NMR, δ: 4.64 (br d, J = 10.4 Hz, H-2), 4.57 (br s, H-3), 4.39 (br s, H-5), 3.57 (s, OMe), 3.53 (br s, H-6), 3.50 (br s, H-1), 2.25 (d, J = 4.4 Hz, HO-C(5)), 1.84 (d, J = 10.4 Hz, HO-C(2)). ESIMS (+) m/z 181 $[M + Na]^+$, 159 $[M + H]^+$.

5-*O*-5′-Azidopentanoyl Ester of *epi*-Sphaeropsidone (15). To compound **2** (5.0 mg) dissolved in anhydrous CH_2Cl_2 (4.5 mL) and pyridine (100 mL) were added DCC (5.0 mg) and 5-azidopentanoic acid (20 μL). The reaction was left at 0 °C for 1 h and then at room temperature for 2 h. The reaction was stopped by evaporation under N_2. The residue (10.0 mg) was purified by TLC on silica gel eluted with $CHCl_3$/*i*-PrOH (95:5, v/v) giving derivatives **15** (7.90 mg, R_f

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

0.74) (Scheme 2) as uncolored oil. **15** had: UV λ_{max} (log ε) 249 (3.48) nm. IR ν_{max} 2098, 1749, 1671, 1624 cm^{-1}. ^1H NMR, 5.92 (br s, H-5), 5.36 (br s, H-3), 3.70 (s, OMe), 3.59 (br s, H-6), 3.45 (br s, H-1), 3.31 (t, J = 6.5 Hz, CH_2-5′), 2.45 (t, J = 7.2 Hz, CH_2-2′), 1.80−1.72 (m, CH_2-3′), 1.68−1.71 (m, CH_2-4′). ESIMS (+) m/z 304 $[M + Na]^+$, 282 $[M + H]^+$.

Seed Conditioning. The haustorium-inducing activity of sphaeropsidones was tested on parasitic seedlings, and the starting material for the haustorium bioassays were parasitic seeds. *Striga* and *Orobanche* seeds require chemical stimulation to germinate but prior they become responsible to germination-inducing factors these seeds require a period of warm stratification called conditioning.[28] To achieve that, *O. crenata*, *O. cumana*, and *S. hermonthica* seeds were surface sterilized by immersion in 0.5% (w/v) NaOCl and 0.02% (v/v) Tween 20, were sonicated for 2 min, rinsed thoroughly with sterile distilled water, and dried in a laminar air flow cabinet. To allow seed conditioning, approximately 100 seeds of each species were placed separately in 9 mm diameter glass fiber filter paper disks (GFFP) moistened with 50 μL of sterile distilled water and placed inside Petri dishes in incubators during 14 days at 30 °C for *S. hermonthica* and 10 days at 22 °C for *O. crenata* and *O. cumana*.

Haustorium-Inducing Assay. A solution of the germination stimulant GR24[29] was prepared at 10^{-6} M in sterile distilled water. Immediately before use, stock solutions in methanol of the candidate haustorial-inducing compounds as sphaeropsidone, **1**, *epi*-sphaeropsidone, **2**, their natural analogues, **3** and **4**, and hemisynthetic derivatives, **5**−**15**, were diluted in the GR24 solution at five decreasing concentrations of sphaeropsidone (100, 10, 1, 0.1, 0.01 μM) on *Striga hermonthica* and three decreasing concentrations of sphaeropsidone (100, 10, 1 μM) on *Orobanche* spp. but keeping constant the concentration of GR24 and methanol in order to allow comparisons. Final concentration of methanol was adjusted to 0.70% (v/v). In these conditions, the solutions of natural sphaeropsidones and derivatives had a pH value of 6.5. GFFP disks containing the conditioned *Striga* and *Orobanche* seeds were transferred inside a laminar flow cabinet to sterile filter paper in order to remove the excess of water used for seed conditioning and then transferred to new 10 cm sterile Petri dishes. Then, each disk was treated with a 50 μL aliquot of the respective test solution. GR24 solution (containing 0.70% methanol) was used as negative control. Seeds were incubated in the dark at 30 °C (*S. hermonthica* seeds) or at 22 °C (*O. crenata* and *O. cumana* seeds). Because of the faster germination of *Striga* when compared with *Orobanche* species, haustorium formation was examined at 3 days in *S. hermonthica* and 6 days in *O. crenata* and *O. cumana*. Using a stereoscopic microscope at 30× magnification, the percentage of haustorium formation in emerged radicles of each species was established.

Statistical Analysis. The bioassays were performed twice with three replicates. Percentage data were approximated to normal frequency distribution by means of angular transformation ($180/\pi \times$ arcsine (sqrt[%/100]) and subjected to analysis of variance (ANOVA) using SPSS software for Windows, version 21.0 (SPSS Inc., Chicago, Illinois, USA). The significant of mean differences between each treatment against the negative control was evaluated by the two-sided Dunnett test. Null hypothesis was rejected at the level of 0.05.

RESULTS AND DISCUSSION

Radicles of *O. crenata* and *O. cumana* did not develop haustorium when treated with the negative control test solution GR24 (Figure 2C,E). This finding is in agreement with observations of radicles made by Cimmino et al. (2014)[21] but in contradiction with the generalized view suggesting that radicle of *Orobanche* seedlings spontaneously initiate typical attachment organs in the absence of exogenous signals.[8−10] *S. hermonthica* radicles exposed to negative control also lacked haustorial development (Figure 2A). The spontaneous development of terminal haustorium does not make biological sense for obligated parasitic weeds such as *Orobanche* and *Striga* as it will threaten the success of host attack. Because the root meristem does not resume its normal growth after terminal haustorium development, a haustorium initiated before host contact impedes successful host attack, leading to the death of the parasitic seedling. In this work instead of spontaneous haustorium development, specificity for the chemistry that initiates terminal haustorium development was expected and was confirmed for *Orobanche* as well as is the case for *Striga*.[30]

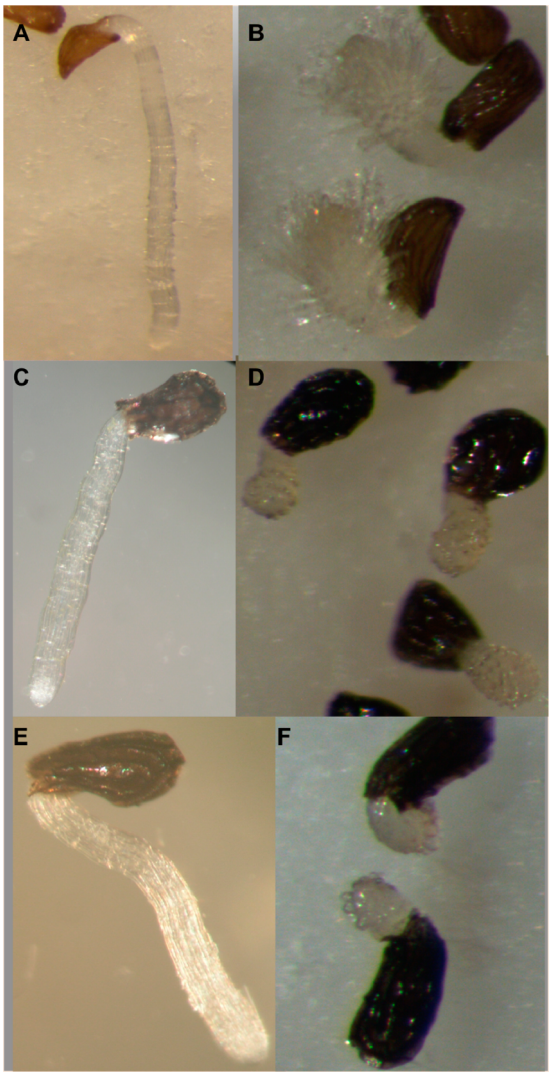


Figure 2. Haustorium formation in (A) *Striga hermonthica* seedlings exposed to negative control, (B) *S. hermonthica* seedlings exposed to sphaeropsidone, (C) *Orobanche crenata* seedlings exposed to negative control, (D) *O. crenata* seedlings exposed to sphaeropsidone, (E) *Orobanche cumana* seedlings exposed to negative control, (F) *O. cumana* seedlings exposed to sphaeropsidone.

Sphaeropsidone and *epi*-sphaeropsidone, phytotoxins produced by *D. cupressi*,[26] were strong inducers of haustoria in *S. hermonthica* radicles. Recognition of sphaeropsidones by radicles of *S. hermonthica* promotes a cessation in the growth of the parasitic radicle and the development of attachment organ at the radicle tips that is in agreement with the description of terminal haustorium made by Riopel and Timko (1995).[4] More than 80% of *S. hermonthica* radicles developed haustorium when exposed to sphaeropsidone at concentrations between 1 and 0.1 μM or to *epi*-sphaeropsidone at concentration between 10 and 1 μM (Figures 2B and 3). For

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

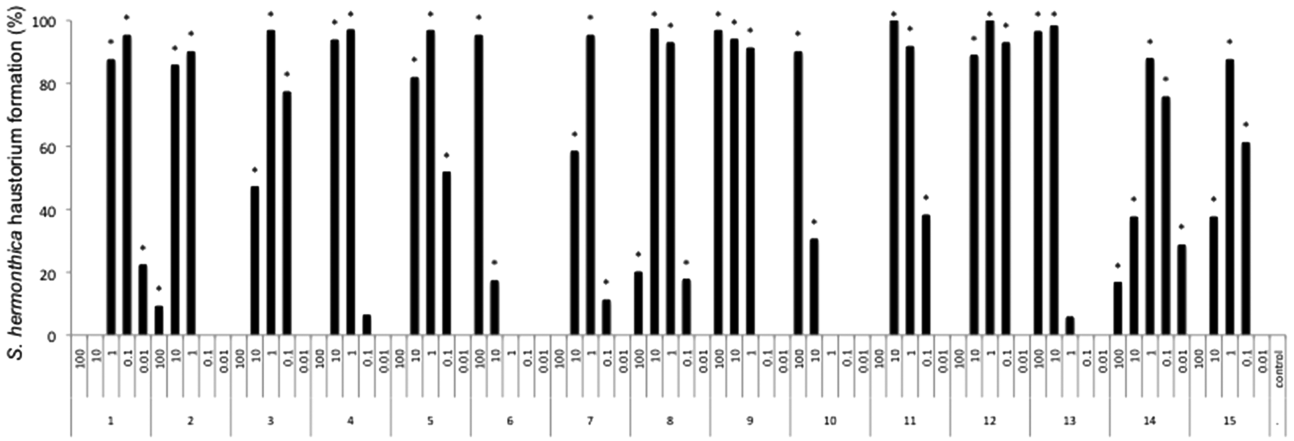


Figure 3. Induction of *S. hermonthica* haustorium formation by sphaeropsidone, **1**, *epi*-sphaeropsidone, **2**, two natural analogues, **3** and **4**, eight sphaeropsidones derivatives, **5**−**10**, **12**, and **14**, and three *epi*-sphaeropsidone derivatives, **11**, **13**, and **15**, tested in the range 0.01−100 μM. *Indicates differences at the 0.05 level compared with the control.

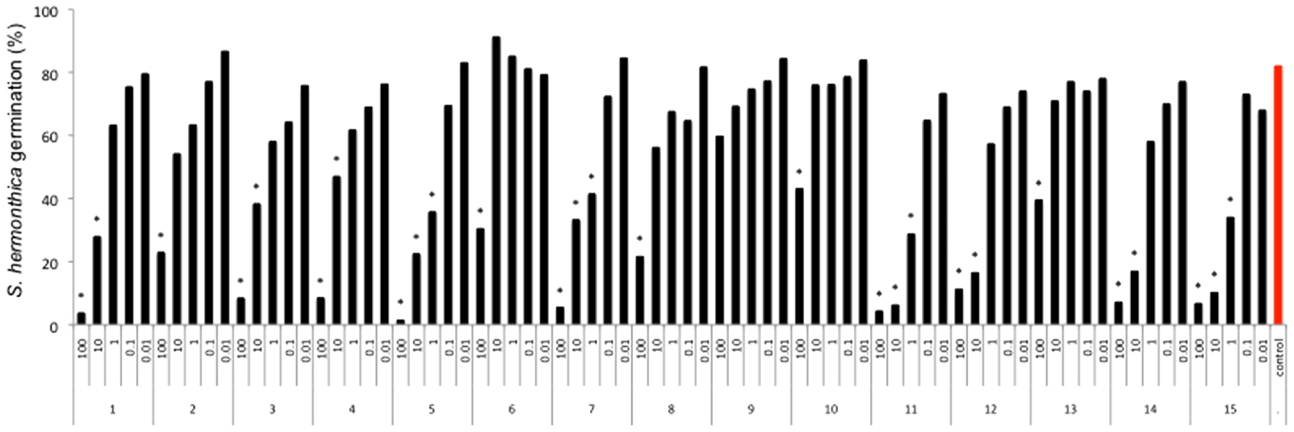


Figure 4. Inhibition of *S. hermonthica* germination by sphaeropsidone, **1**, *epi*-sphaeropsidone, **2**, two natural analogues, **3** and **4**, eight sphaeropsidones derivatives, **5**−**10**, **12**, and **14**, and three *epi*-sphaeropsidone derivatives, **11**, **13**, and **15**, tested in the range 0.01−100 μM. *Indicates differences at the 0.05 level compared with the control.

each compound, decreased concentrations did not induce *S. hermonthica* haustorium while higher concentrations showed inhibition activity of *S. hermonthica* germination (Figure 4).

Necrosis was not detected in *S. hermonthica* radicles during the length of the experiment at any concentration. Both phytotoxic and organogenic activities in radicles of parasitic weeds has been previously observed for haustorium-inducing factors.[31] Both active concentrations of sphaeropsidone, 1 and 0.1 μM, induced similar rate of haustorium induction (85% of radicles developed haustorium) (Figure 3). However, the radicles grew longer before detecting the haustorium-inducing signal at 0.1 than at 1 μM, visualized by longer radicles terminated in haustorium (data not shown). The same phenomenon was observed with *epi*-sphaeropsidone as *S. hermonthica* radicles terminated in haustorium were longer when treated at 1 μM than at 10 μM. This could be indicating that the required time of exposure for signal detection and haustorium development was indirectly related with the compound concentration. Parasitic radicle responding later to decreased concentrations of haustorium-inducing signal could constitute a mechanism aimed to measure distance for host attachment.[30] Several phenolic derivatives of plant origin have been previously identified as haustorium-inducing factors for *Striga*.[4,15,30] Many of those phytochemicals are commonly found in the rhizosphere and play roles in chemical communication with microbial organisms. Fungal phytotoxins have been described previously with potential to trigger host-dependent developmental programs in parasitic plants such as germination[20,21] but never were related before to the chemistry of haustorium initiation. Active concentrations of **1** and **2** where similar to those performed by previously known haustorium-inducing factors such as xenognosin A and B, 2,6-dimethoxybenzoquinone, ferulic, vanillic, sinapic, and syringic acids.[15,30,32]

Radicles of *O. crenata* and *O. cumana* responded to sphaeropsidone and *epi*-sphaeropsidone with a cessation of radicle elongation, the radicle tip became spherical, and crowns of cell extensions in the form of papillae were observed at the outer surface around the apex (Figure 2D,F). This observation is in agreement with the description of anchoring device of *Orobanche* haustoria made by Joel and Losner-Goshen (1994).[8] According with *S. hermonthica* results, *epi*-sphaeropsidone was a weaker *Orobanche* haustorium-inducer than sphaeropsidone. These results may suggest that the haustorial activity of sphaeropsidones is mediated by a similar mechanism in *Striga* and *Orobanche*. The activity of both sphaeropsidones in *Orobanche* radicles was slightly weaker than that observed in *Striga* radicles. Haustorium was observed in *Orobanche* radicles

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

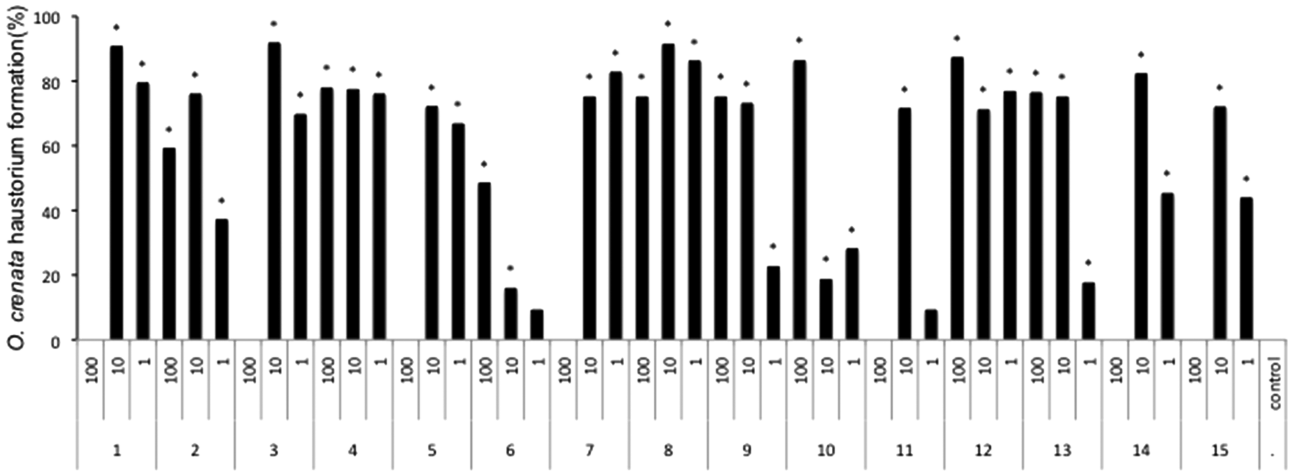


Figure 5. Induction of *O. crenata* haustorium formation by sphaeropsidone, **1**, *epi*-sphaeropsidone, **2**, two natural analogues, **3** and **4**, eight sphaeropsidones derivatives, **5**−**10**, **12**, and **14**, and three *epi*-sphaeropsidone derivatives, **11**, **13**, and **15**, tested in the range 1−100 μM.. *Indicates differences at the 0.05 level compared with the control.

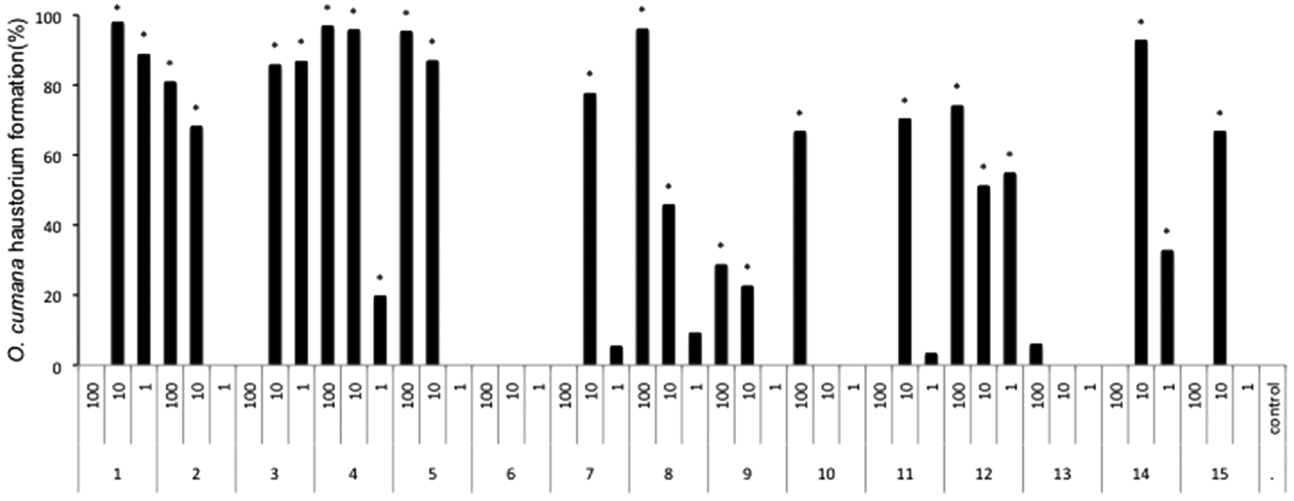


Figure 6. Induction of *O. cumana* haustorium formation by sphaeropsidone, **1**, *epi*-sphaeropsidone, **2**, two natural analogues, **3** and **4**, eight sphaeropsidones derivatives, **5**−**10**, **12**, and **14**, and three *epi*-sphaeropsidone derivatives, **11**, **13**, and **15**, tested in the range 1−100 μM. *Indicates differences at the 0.05 level compared with the control.

treated with sphaeropsidone at concentrations between 10 and 1 μM and with *epi*-sphaeropsidone at concentrations between 100 and 10 μM (Figures 5 and 6). The induction of haustorium development by exogenous signals has never been documented for *Orobanche*, and therefore our results constitute a breakthrough against the general belief that *Orobanche* does not respond to exogenous stimulation in order to develop haustoria.[8−10] Sphaeropsidone at 100 μM did not inhibit *Orobanche* germination but it was phytotoxic, inhibiting *Orobanche* radicle development leading to haustorium inhibition (Figures 5 and 6). The inhibitory activity developed by sphaeropsidones at high concentrations differentially targeted the processes of germination in *Striga* and radicle development in *Orobanche*. Phytotoxicity developed by haustorium inducing factors has been previously associated with a common metabolic pathway shared by germination and root growth processes.[5]

These results prompted to prepare some derivatives of both **1** and **2** to elucidate patterns in the molecular specificity of haustorial induction in *S. hermonthica*, *O. crenata*, and *O. cumana*. *epi*-Sphaeropsidone, **2** (Figure 1), is a natural isomer of **1** (Figure 1), being its epimer at C-5, and the chlorosphaeropsidones **3** and **4** (Figure 1) are two C-6 epimeric natural analogues of sphaeropsidones showing the opening of the epoxy ring and its conversion in the corresponding chloridrin, with the chlorine at C-6 and the hydroxy group at C-5.

Six already known derivatives of sphaeropsidone (**5**−**10**, Scheme 1) and one of *epi*-sphaeropsidone (**11**, Scheme 2) were hemisynthesized and showed the same spectroscopic properties previously reported.[26] Derivative **5** showed the reversible acetylation of the hydroxy group at C-5, while the 2,4,5-acetoxyanisole, **6**, obtained from the same acetylation reaction, is a tetrasubstituted benzene derivative and thus also the aromatization of the cyclohexenone ring. The same hydroxy group at C-5 was converted by oxidation to carbonyl group, yielding the corresponding 2,3-epoxyhemiquinone **7**, while the reduction of the carbonyl group at C-2 generated the corresponding 3-methoxy-cyclohex-3-ene-2,5-diol **9**. **1** was also converted into the corresponding bromydrin **8**, which differs from the epichlorosphaeropsidone **4** for the halogen at C-1, which has a bromine instead of chlorine, and for the opposite stereochemistry of the hydroxy group at C-5. The

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

catalytic hydrogenation of **1** yielded 2,4-dihydroxy-5-methoxycyclohexanone **10**, which showed the saturation of the double bond between C-2 and C-3 and the reductive opening of the epoxy ring. *epi*-Sphaeropsidone, **2**, was converted into the corresponding acetyl derivative **11**, which, although epimer at C-5, showed as **5** the reversible esterification of the hydroxy group at the same carbon.

Sphaeropsidone, **1** was converted into the new esters **12** and **14** (Scheme 1), which showed always the reversible modification of the hydroxy group at C-5 although the acyl group is different, being a *p*-Br-benzoyl in **12** and 5-azidopentanoyl in **14**. Both derivatives **12** and **14** showed spectroscopic properties consistent with their structures. Significantly, their IR spectra did not show bands for hydroxy groups.[33] In particular, the ^{1}H NMR spectrum of **12** differed from that of **1** for the expected downfield shift ($\Delta\delta$ 0.96) of H-5 appearing as a doublet (J = 2.5 Hz) and for the couple of two doublets (J = 8.7 Hz) of the protons of the *para*-substituted benzene ring resonating at δ 8.01 and 7.63 for H-2′,6′ and H-3′,5′, respectively. Its ESIMS spectrum showed the characteristic isotopic peaks of bromine for both sodium clusters $[M + 2 + Na]^+$ and $[M + Na]^+$ and the pseudomolecular ion $[M + 2 + H]^+$ and $[M + H]^+$ at m/z 364 and 362, and 342 and 340, respectively. The ^{1}H NMR spectrum of **14** differed from that of **1** for the expected downfield shift ($\Delta\delta$ 0.74) of H-5, which resonated as a doublet (J = 2.9 Hz) at δ 5.97, and for the presence of the signals of the 5-azidopentanoyl group appearing as two triplets (J = 6.5 and 7.3 Hz, respectively) at δ 3.32 and 2.53 for the methylene protons, respectively, linked to the azido and the carboxyl group. The other two methylene groups appeared as multiplets at 1.83−1.76 (H_2C-3′) and 1.71−1.64 (H_2C-4′). Its ESIMS spectrum showed the sodium cluster $[M + Na]^+$ and the pseudomolecular ion $[M + H]^+$ at m/z 304 and 282, respectively.

epi-Spheropsidone, **2**, was chemically reduced into the corresponding 3-methoxy-cyclohex-3-ene-2,5-diol, **13** (Scheme 2), which is an epimer at C-5 of **9**, similarly obtained from **1**. The ^{1}H NMR spectrum of **13** differed from that of **2** for the significant presence of the broad doublet (J = 10.4 Hz) of H-2 and for the expected upfield shift ($\Delta\delta$ 0.66) of H-3, which appeared as a broad singlet at δ 4.57 instead of a doublet. Its ESIMS spectrum showed the sodium cluster $[M + Na]^+$ and the pseudomolecular ion $[M + H]^+$ at m/z 182 and 159, respectively. As **1** was converted into **14**, **2** was similarly esterified into the corresponding 5-azidopentanoyl derivative **15** (Scheme 2). Its IR spectrum did not show a band for a hydroxy group. The ^{1}H NMR spectrum of **15** differed from that of **2** for the expected downfield shift ($\Delta\delta$ 1.28) of H-5, which appeared as a broad singlet at δ 5.92 and for the presence of the signals of the 5-azidopentanoyl group appearing as two triplets (J = 6.5 and 7.2 Hz, respectively) at δ 3.31 and 2.45 for the methylene protons, respectively, linked to the azido and the carboxyl group. The other two methylene protons appeared both as multiplets at δ 1.80−1.72 (H2C-3′) and 1.71−1.68 (H2C-4′). Its ESIMS spectrum showed the sodium cluster $[M + Na]^+$ and the pseudomolecular ion $[M + H]^+$ at m/z 304 and 282, respectively.

The haustorium-inducing activity in *S. hermonthica* of sphaeropsidones (**1**−**4**) and their derivatives (**5**−**15**) is shown in Figure 3. The *S. hermonthica* assays indicated that conversion of **1** in derivatives **6** and **10** led to a severe reduction in the haustorium-inducing activity being only significant at 100 μM, while conversion in derivatives **4**, **7**, **8**, and **9** led to loss at 0.1 μM of haustorium-inducing activity that characterizes the parent compound. Structural modifications in **3**, **5**, **12**, and **14** did not affect the haustorial activity, inducing high rates of *S. hermonthica* haustoria at 1 and 0.1 μM comparable with sphaeropsidone, **1**.

Esterification at C-5 in **11** and **15** increased haustorium-inducing activity on *S. hermonthica* seedlings in comparison with **2**, and in derivative **14** in comparison with **1**, while the modifications at C-5 made in **3** and **12** maintained the strong activity observed in **1** up to 0.1 μM. Acetylation of **1** in derivatives **5** did not strongly affect the haustorial activity as **1** and **5** are strong inducers, although a slight reduction in activity was found at 0.1 μM in derivative **5** in comparison with **1**. It is interesting to note that **2** and derivative **11** are weaker haustorial inducers when compared with their respective epimers of **1** and **5**. These results allow us to hypothesize that the stereochemistry at C-5, although not strongly, play an important role in impart this activity.

The epoxy ring by itself was not essentially associated with haustorial inducing activity as some derivatives lacking the epoxy ring, i.e., the analogue **3**, had strong haustorial activity, however, when the selective reductive opening of the epoxy ring was associated with the reduction of the olefinic double bond in **10** or with the aromatization of the cyclohexenone ring in **6**, the haustorial-inducing activity was strongly affected. The reduction of the C-2 carbonyl group in **9** and **13** led to reduction of haustorial activity in comparison with their parent compounds **1** and **2** in seedlings.

These results could be explained, except for **7**, in the easy conversion of all derivatives at physiological pH into sphaeropsidone and then into the corresponding above hypothesized 2,3-epoxy-5-methoxyquinone. In fact, **3**, having the requested stereochemistry of the hydroxy and chlorine groups at C-5 and C-6, according to the well-known intramolecular nucleophilic substitution could give **1**. This reaction is less easy for the other chloridrin **4**, probably for the opposite not suitable stereochemistry of chlorine at C-6.[34] Furthermore, derivatives **5**, **12**, and **14** being ester of **1** at physiological pH, according to the well-known lethal metabolism,[35] probably were hydrolyzed into **1**. The slight minor activity of derivative **8** is due to the same reasons above-reported for **4** having the bromine in **8** the same stereochemistry of chlorine in **4**. The reduced activity of **9** could be explained from different oxidation stage in respect to **1** being **9**, a 3-methoxy-cyclohex-3-ene-2,5-diol, and **1**, a 2,3-epoxy-5-methoxyhemiquinone. The noteworthy reduction of the activity of derivatives **6** and **10** was due to the inability to convert their benzene and the cyclohexanone rings into 2,3-epoxyhemiquinone ring of sphaeropsidone and then in the corresponding 2,3-epoxy-5-methoxyyquinone.

The haustorium-inducing activity in *O. crenata* and *O. cumana* of sphaeropsidones (**1**−**4**) and their derivatives (**5**−**15**), is shown in Figures 5 and 6, respectively. SAR results indicated the haustorium-inducing activity of the parent compound was affected by the structural modifications in a slightly different way for *Striga* and *Orobanche* species, being those differences more obvious between *Striga* and *O. cumana*. The reduction of C-2 carbonyl group in **9** and **13** led to reduction of haustorial activity at 1 μM in *O. crenata* seedlings, but it induced a strong loss of activity at all concentrations in *O. cumana*. A marked loss of haustorial-inducing activity was observed in *O. crenata* for compounds **6** and **10** when compared with **1**, as it was observed for *S. hermonthica*, while

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

the activity was completely lost for *O. cumana*. For *O. crenata*, structural modifications in compounds **5** and **12** maintained nearly the same levels of haustorial activity than **1**, as it happened for *S. hermonthica*, however, activity of compound **5** was lost at 1 μM for *O. cumana*. The activity of compounds **4** and **8** was observed to be very high for *O. crenata*, being the haustorial papillae especially well developed by compound **4** in both *Orobanche* species. Differential effects caused by structural modifications across hemiparasitic genera have been previously suggested. Steffens et al. (1982)[32] found that for the system *Agalinis purpurea*−xenognosin A, haustorium was initiated when the molecular inducer meet two structural features: (i) hydroxy and methoxy groups were present and both groups occupying a *meta*-position and (ii) an alkyl branching *ortho* to the methoxy substituent. MacQueen (1984)[15] studied the activity of phenolic compounds on *S. hermonthica* and demonstrated that the activity was also initiated when the hydroxy group was occupying a *para*-position and the methoxy group a *meta*-position while the alkyl branching *ortho* to the methoxy substituent was not required for activity in *Striga*.

Regarding germination inhibition, the activity of the two natural analogues (**3** and **4**), eight sphaeropsidones derivatives (**5**−**10**, **12**, and **14**), and three *epi*-sphaeropsidone derivatives (**11**, **13**, and **15**) in comparison with sphaeropsidone, **1**, and *epi*-sphaeropsidone, **2**, was tested on *S. hermonthica* (Figure 4). The germination inhibitory activity was reduced in **6**, **8**, **9**, and **10** and increased in **5**, **7**, **12**, and **14** in comparison with sphaeropsidone, **1**, while it was reduced in **13** and increased in **11** and **15** in comparison with *epi*-sphaeropsidone, **2**. The increased germination inhibitory activity in **5**, **11**, **12**, **14**, and **15** can be explained by their increased lipophilicity which could facilitate cell membrane permeability.[35] The reduced germination inhibitory activity in **9** and **13** could be explained for the absence of the α,β-unsaturated ketone due to the reduction of the carbonyl group at C-2, and in **6** and **10** for the aromatization of the cyclohexenone ring, and for the reduction of the double bond between C-4 and C-5 and for the reductive opening of the epoxy ring.

The SAR study results suggested that the ability to initiate haustorium development in *S. hermonthica* is probably due to the conversion of the natural sphaeropsidones, their analogues, and hemisynthetic derivatives in the corresponding 4-methoxyquinone. This conversion could occur more easily in the analogue **3** and derivatives **5**, **8**, **12**, and **14** of sphaeropsidone and derivatives **11** and **15** of *epi*-sphaeropsidones. In fact, as above cited the esters **5**, **11**, **12**, and **14**, by hydrolysis, could be converted into the parent compounds as well as the analogue **3** and derivative **8**, by nucleophilic elimination.[36] A successive oxidation step could be converted both **1** and **2** into the 2,3-epoxy-4-methoxyquinone, and this finally, by reductive opening of the epoxy group followed by water nucleophilic elimination into the 4-methoxyquinone. This hypothesis on the mode of action of sphaeropsidone is in full agreement with the results obtained using natural and synthetic quinones as sorghum xenognosin and dimethoxybenzoquinones, the latter very closely related to the 4-methoxyquinone, which as above explained could be generated by oxidation of sphaeropsidones in the studies carried out on haustoria and the chemistry in host recognition ion parasitic angiosperms. Quinone/hydroquinone structures serve as cofactors in many metabolic pathways, playing critical chemical roles in oxidation/reduction processes.[31,36] This mode of action could also operate in the haustorium-induction in *Orobanche*. The slight difference observed between *Orobanche* and *Orobanche* is not surprising as differences between the species in their ability to recognize and respond to the same haustorium-inducing factors has been reported.[31] The presence of the epoxy ring and the α,β-unsaturated ketone group could be the responsible for the inhibition of *S. hermonthica* seed germination.

The induction of haustorium is a little researched area in parasitic weeds, especially in *Orobanche* species. Our work has identified for the first time natural occurring compounds with haustorium-inducing activity for *Orobanche* species, which opens several research directions aimed either to understand the chemistry of haustorium initiation or toward the development of alternatives to conventional control. It is now possible to envision research in which host-encoded haustorium-inducing factors are identified through bioactivity-guided chromatographic purification of host root extracts. In addition, *Orobanche* transgenic seedlings in which candidate genes for haustorium initiation are silenced could be easily phenotyped by the in vitro application of haustorium inducers. It could be possible, in addition, once host-encoded haustorium factors are identified for *Orobanche*, to select crop cultivars with low haustorium-induction activity in their roots. In addition, this work also opens the way of the development of new agrochemicals with a novel mode of action based on disturbing the fine-tuned process of host recognition. Compounds such as sphaeropsidones with the ability of triggering haustorium induction in a suicidal fashion are good candidates for such strategy. Current research in our laboratory is starting to explore the above-mentioned alternatives for *Orobanche* weed management.

AUTHOR INFORMATION

Corresponding Author

*Phone: +39 081 2539178. Fax: +39 081 674330. E-mail: evidente@unina.it.

Funding

The work was carried out in the frames of COST Action FA1206 Strigolactones: biological roles and applications. M. Fernández-Aparicio was supported by the European Union, Marie-Curie FP7 COFUND People Programme, through the award of an AgreenSkills fellowship (under grant agreement no. PCOFUND-GA-2010-267196) with additional support from INRA Division "Santé des Plantes et Environment, appel à projets scientifiques 2015_Catégorie Biocontrôle".

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the "Istituto di Chimica Biomolecolare del CNR", Pozzuoli, Italy, for recording NMR spectra. Antonio Evidente is associated with "Istituto di Chimica Biomolecolare del CNR", Pozzuoli, Italy.

REFERENCES

(1) Parker, C.; Riches, C. R. *Parasitic Weeds of the World: Biology and Control*; CAB International: Wallingford, UK, 1993.

(2) Heide-Jørgensen, H. S. Introduction: the parasitic syndrome in higher plants. In *Parasitic Orobanchaceae*; Joel, D. M., Gressel, J., Musselman, L. J., Eds.; Springer: Dordrecht, The Netherlands, 2013; pp 1−23.

(3) Kuijt, J. *The Biology of Parasitic Flowering Plants*; University of California Press: Berkeley, 1969.

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(4) Riopel, J. L.; Timko, M. P. Haustorial initiation and differentiation. In *Parasitic Plants*; Press, M. C., Graves, J. D., Eds.; Chapman and Hall: London, 1995; pp 39−79.
(5) Tomilov, A.; Tomilova, N.; Shin, D. H.; Jamison, D.; Torres, M.; Reagan, R.; McGray, H.; Horning, T.; Truong, R.; Nava, A. J.; Nava, A.; Yoder, J. I. Chemical signaling between plants: mechanistic similarities between phytotoxic allelopathy and host recognition by parasitic plants. In *Chemical Ecology: From Gene to Ecosystem*; Dicke, M., Ed.; Springer: Dordrecht, The Netherlands, 2006; pp 55−69.
(6) Fernández-Aparicio, M.; Kisugi, T.; Xie, X.; Rubiales, D.; Yoneyama, K. Low strigolactone root exudation: a novel mechanism of broomrape (*Orobanche* and *Phelipanche* spp.) resistance available for faba bean breeding. *J. Agric. Food Chem.* **2014**, *62*, 7063−7071.
(7) Westwood, J. H.; Yoder, J. I.; Timko, M. P.; de Pamphilis, C. W. The evolution of parasitism in plants. *Trends Plant Sci.* **2010**, *15*, 227−235.
(8) Joel, D. M.; Losner-Goshen, D. The attachment organ of the parasitic angiosperms *Orobanche cumana* and *O. aegyptiaca* and its development. *Can. J. Bot.* **1994**, *72*, 564−574.
(9) Joel, D. M. Direct infection of potato tubers by the root parasite *Orobanche aegyptiaca*. *Weed Res.* **2007**, *47*, 276−279.
(10) Bandaranayake, P. C. G.; Yoder, J. I. Haustorium initiation and early development. In *Parasitic Orobanchaceae*; Joel, D. M., Gressel, J., Musselman, L. J., Eds.; Springer: Dordrecht, The Netherlands, 2013; pp 61−74.
(11) Cimmino, A.; Fernández-Aparicio, M.; Andolfi, A.; Basso, S.; Rubiales, D.; Evidente, A. Effect of fungal and plant metabolites on broomrapes (*Orobanche* and *Phelipanche* spp.) seed germination and radicle growth. *J. Agric. Food Chem.* **2014**, *62*, 10485−10492.
(12) Cimmino, A.; Masi, M.; Evidente, M.; Superchi, S.; Evidente, A. Fungal phytotoxins with potential herbicidal activity: Chemical and biological characterization. *Nat. Prod. Rep.* **2015**, *32*, 1629−1653.
(13) Albrecht, H.; Yoder, J. I.; Phillips, D. A. Flavonoids promote haustoria formation in the root parasite *Triphysaria versicolor*. *Plant Physiol.* **1999**, *119*, 585−592.
(14) Smith, C. E.; Ruttledge, T.; Zeng, Z. X.; O'Malley, R. C.; Lynn, D. G. A mechanism for inducing plant development: the genesis of a specific inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 6986−6991.
(15) McQueen, M. Haustorial initiating activity of several simple phenolic compounds, In *Proceedings of the Third International Symposium on Parasitic Weeds*. ICARDA: Aleppo, Syria, 1984; pp 118−122.
(16) Atsatt, P. R. The insect herbivore as a predictive model in parasitic seed plant biology. *Am. Nat.* **1977**, *111*, 579−586.
(17) Keyes, W. J.; Taylor, J. V.; Apkarian, R. P.; Lynn, D. G. Dancing together. Social controls in parasitic plant development. *Plant Physiol.* **2001**, *127*, 1508−1512.
(18) Bandaranayake, P. C. G.; Filappova, T.; Tomilov, A.; Tomilova, N. B.; Jamison-McClung, D.; Ngo, Q.; Inoue, K.; Yoder, J. I. A single-electron reducing quinone oxidoreductase is necessary to induce haustorium development in the root parasitic plant *Triphysaria*. *Plant Cell* **2010**, *22*, 1404−1419.
(19) Capasso, R.; Evidente, A.; Cutignano, A.; Vurro, M.; Zonno, M. C.; Bottalico, A. Fusaric and 9,10-dehydrofusaric acids and their methyl esters from *Fusarium nygamai*. *Phytochemistry* **1996**, *41*, 1035−1039.
(20) Yoneyama, K.; Takeuchi, Y.; Ogasawara, M.; Konnai, M.; Sugimoto, Y.; Sassa, T. Cotylenins and fusicoccins stimulate seed germination of *Striga hermonthica* (Del.) Benth and *Orobanche minor* Smith. *J. Agric. Food Chem.* **1998**, *46*, 1583−1586.
(21) Evidente, A.; Andolfi, A.; Fiore, M.; Boari, A.; Vurro, M. Stimulation of *Orobanche ramosa* seed germination by fusicoccin derivatives: A structure-activity relationship study. *Phytochemistry* **2006**, *67*, 19−26.
(22) Fernández-Aparicio, M.; Andolfi, A.; Cimmino, A.; Rubiales, D.; Evidente, A. Stimulation of seed germination of *Orobanche* species by ophiobolin A and fusicoccin derivatives. *J. Agric. Food Chem.* **2008**, *56*, 8343−8347.
(23) Sparapano, L.; Bruno, G.; Fierro, O.; Evidente, A. Studies on structure-activity relationship of sphaeropsidins A-F, phytotoxins produced by *Sphaeropsis sapinea* f. sp. cupressi. *Phytochemistry* **2004**, *65*, 189−198.
(24) Evidente, A.; Sparapano, L.; Fierro, O.; Bruno, G.; Giordano, F.; Motta, A. Sphaeropsidone and episphaeropsidone, phytotoxic dimedone methyl ethers produced by *Sphaeropsis sapinea* f. sp. *cupressi* grown in liquid culture. *Phytochemistry* **1998**, *48*, 1139−1143.
(25) Evidente, A.; Sparapano, L.; Andolfi, A.; Bruno, G.; Giordano, F. Chlorosphaeropsidone and epichlorospaeropsidone, two chlorinated dimedone methyl ether isolated from liquid cultures of *Sphaeropsis sapinea* f. sp. *cupressi*. *Phytopathol. Mediterr.* **2000**, *39*, 299−309.
(26) Evidente, A.; Maddau, L.; Scanu, B.; Andolfi, A.; Masi, M.; Motta, A.; Tuzi, A. Sphaeropsidones, phytotoxic dimedone methyl ethers produced by *Diplodia cupressi*: A structure-activity relationship study. *J. Nat. Prod.* **2011**, *74*, 757−763.
(27) Berger, S., Braun, S. *200 and More Basic NMR Experiments: A Practical Course*, 1st ed.; Wiley-VCH: Weinheim, 2004.
(28) Lechat, M. M.; Pouvreau, J. B.; Péron, T.; Gauthier, M.; Montiel, G.; Veronesi, C.; Todoroki, Y.; Le Bizec, B.; Monteau, F.; Macherel, D.; Simier, P.; Thoiron, S.; Delavault, P. PrCYP707A1, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analogue GR24. *J. Exp. Bot.* **2012**, *63*, 5311−5322.
(29) Johnson, A. W.; Rosebery, G.; Parker, C. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Res.* **1976**, *16*, 223−227.
(30) Chang, M.; Lynn, D. G. The haustorium and the chemistry of host recognition in parasitic angiosperms. *J. Chem. Ecol.* **1986**, *12*, 561−579.
(31) Jamison, D. S.; Yoder, J. I. Heritable variation in quinone-induced haustorium development in the parasitic plant *Triphysaria*. *Plant Physiol.* **2001**, *125*, 1870−1879.
(32) Steffens, J. C.; Lynn, D. G.; Kamat, V. S.; Riopel, J. L. Molecular specificity of haustorial induction in *Agalinis purpurea* (L.) Raf. (Scrophulariaceae). *Ann. Bot.* **1982**, *50*, 1−7.
(33) Nakanishi, K.; Solomon, P. H. *Infrared Absorption Spectroscopy*, 2nd ed.; Holden Day: Oakland, CA, 1977; pp 17−44.
(34) Carey, F. A.; Sandberg, R. J. *Advanced Organic Chemistry. Part B, Reactions and Synthesis*; Kluver Academic/Plenum Publisher: New York, 2001; pp 152.
(35) Hassal, K. A. *Biochemistry and Use of Pesticides*; Verlag Chemie: Weinheim, Germany, 1990; pp 58, 72, 304, 429, and 497.
(36) Palmer, A. G.; Gao, R.; Maresh, J.; Erbil, J. W.; Lynn, D. G. Chemical and biology of multi-host/pathogen interactions.: chemical perception and metabolic complementation. *Annu. Rev. Phytopathol.* **2004**, *42*, 439−64.

DOI: 10.1021/acs.jafc.6b01910
J. Agric. Food Chem. XXXX, XXX, XXX−XXX