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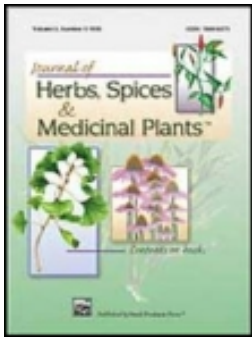
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Evaluation of *Agastache*
and Other Lamiaceae Species
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Roger G. Fuentes-Granados
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ABSTRACT. The reaction of 14 populations of *Agastache* maintained at the North Central Regional Plant Introduction Station to *Verticillium* wilt was evaluated. The plants were inoculated by root dip with 3 strains of *Verticillium dahliae*: peppermint, potato, and *Agastache*. Infected plants were obtained only from inoculation with the *Agastache* strain of *Verticillium*. *Agastache rugosa* had a higher rate of infection (10-21%) than *Agastache foeniculum* and *Agastache nepetoides*, both with infection rates \leq 5 percent. A pathogenicity test

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of the *Agastache* strain of *Verticillium* conducted on various species of the mint family resulted in clear symptoms of disease, such as leaf curling, plant wilting, and death in individuals of *Pycnanthemum pilosum* and *Salvia azurea*. Internal infection with *Verticillium* was also detected in 1 species of *Calamintha* (14%) and 3 species of *Ocimum* (38-54%). Results of this study revealed interspecific variability for susceptibility to *Verticillium* among species of *Agastache*. The possible specificity of the *Agastache* strain of *Verticillium* is also supported by our results. [Article copies available from The Haworth Document Delivery Service: 1-800-342-9678.]

KEYWORDS. Disease evaluation, essential oils, nectar plants.

INTRODUCTION

Species of *Agastache* Clayton ex Gronov., such as *Agastache foeniculum* (Pursh) Kuntze, *Agastache nepetoides* (L.) Kuntze, and *Agastache rugosa* (Fish. & Meyer) Kuntze, have been reported to produce potentially useful essential oils (4). A study conducted by Charles et al. (4) noted that methylchavicol, a common flavoring agent, was present in all 19 lines of *Agastache* tested and was the major oil constituent in most accessions of *Agastache foeniculum* and *Agastache rugosa*. *Agastache foeniculum* is cultivated in Finland for aromatic foliage that is added to tea mixtures, cakes, ices, and sweets, and which is used in home kitchens (9). Several reports (9,10,11,21) indicate that horticulturists in many countries are interested in cultivating *Agastache* as a potential source of aromatic oils and for direct use as a culinary herb.

Agastache also is a potentially valuable source of nectar for honey bees (1). The decline of suitable bee pasture for midwestern beekeepers, as well as a need at the North Central Regional Plant Introduction Station (NCRPIS) to establish special plantings to provide honey bees with nectar throughout the growing season, led Widrechner (22) to establish field plots of perennial mints to evaluate as nectar sources. Results of these evaluations indicated that *Agastache foeniculum* was one of the best species for bee forage in the trial. Mayer et al. (12) described a limited-scale honey bee operation in Washington State in which *Agastache foeniculum* served as the nectar source, and through an economic analysis of the operation, concluded *Agastache foeniculum* could be grown profitably as a nectar source for honey bees.

The cultivation of *Agastache* could be limited by plant susceptibility to

Verticillium wilt (3), a fungal disease caused by at least 3 species of *Verticillium*. This organism has a worldwide distribution in both temperate and tropical regions (17). McKeen (13) has stated that, from a wide range of horticultural crops affected with Verticillium wilt, *Verticillium dahliae* Kleb. was isolated with the highest frequency, 84.3 percent, followed by *Verticillium albo-atrum* Reinke & Berth. and *Verticillium nigrescens* Pe-thyb., which were isolated at much lower frequencies. In a first report of Verticillium wilt in *Agastache rugosa*, Block et al. (3) concluded that the causal agent was *Verticillium dahliae*. Symptoms included wilting, interveinal yellowing of the lower leaves, and internal vascular browning of the stem (3).

Most of the work reported on Verticillium wilt in the Lamiaceae has been conducted in *Mentha* because of the importance of this plant to the domestic aromatic oil industry. Verticillium wilt has caused the abandonment of thousands of the most productive acres in the major mint-growing areas of Michigan and Indiana since a first outbreak in 1924 (16). Resistant varieties of peppermint with commercially acceptable essential oils were successfully obtained only after the exposure of stolons of the susceptible variety, cv. Black Mitcham, to irradiation (15). Reports of Verticillium wilt on Lamiaceae genera other than *Mentha* include those for *Agastache* (3), *Hedeoma* (20), *Lamium* (7), *Monarda* (18), and *Salvia* (2,7). Reports suggesting intraspecific or interspecific variability for the response of these plants to *Verticillium* could not be located.

Because of the potential for Verticillium wilt to limit production of *Agastache* and because little is known about genetic variability for resistance to *Verticillium* among Lamiaceae, an experiment was designed to evaluate the reactions of 14 accessions of *Agastache* to 3 strains of *Verticillium dahliae*. Another experiment, designed to investigate the host range of the *Agastache* strain of *Verticillium*, was conducted with 9 other Lamiaceae species maintained at the NCRPIS.

MATERIALS AND METHODS

Pathogen. Potato and peppermint strains of *Verticillium dahliae* were obtained (accessions 18697 and 11405, respectively) from the American Type Culture Collection (ATCC), Rockville, Maryland. An isolate of *Verticillium dahliae* from *Agastache rugosa* (3) was obtained from Charles Block at the NCRPIS. Fungal cultures were rehydrated in sterile water and plated on a potato dextrose agar amended with 5 percent yeast extract (ATCC, personal communication). Cultures were incubated in the dark at 20 to 25°C for 14 days. Conidial suspensions were prepared by rinsing

conidia off the plates with sterile water. Inoculum density was standardized at 10^5 conidia/ml using a hemocytometer. The pathogenicity of the peppermint and potato strains of *Verticillium* was confirmed by inoculating plants (8) of *Mentha piperita* L. (cv. Black Mitcham) and *Solanum tuberosum* L. (cv. Russet Burbank), respectively, plants reported to be susceptible to *Verticillium dahliae* (14,16). In compliance with restrictions imposed by State and Federal laws, the fungus was handled under controlled greenhouse conditions to avoid escape of the organism to natural environments.

Plants. A number of *Agastache* accessions of different origin were included in the experiment (Table 1). Plants were grown from seeds harvested from plants growing in a field where *Verticillium dahliae* was present (3). To eliminate surface-borne fungal contamination, seeds were immersed for 45 sec in an aqueous solution of 63 percent ethanol, 0.525 percent sodium hypochlorite, and 0.1 percent tergitol NPX (Sigma Chemical Co., St. Louis, MO) (5). Seeds were incubated on moist blotters in germination boxes and pre-chilled at 4°C for a week to improve germination. Immediately after chilling, the germination boxes were placed into a germination chamber with daily temperature and light regimens of 8 h at 20°C in the dark and 16 h at 30°C with fluorescent lighting. After 1 week, seedlings were transplanted into plastic cones containing autoclaved growing medium and placed on a mist bench in the greenhouse. The growing

TABLE 1. Origin of *Agastache* species used in evaluation.

Species	Accession	Origin
<i>Agastache foeniculum</i>	A-10206	Cultivated: Putaway, Poland
<i>Agastache foeniculum</i>	PI-561054	Cultivated: Morden, Manitoba
<i>Agastache foeniculum</i>	PI-561055	Cultivated: Washtenaw Co., MI
<i>Agastache foeniculum</i>	PI-561056	Cultivated: Story Co., IA
<i>Agastache foeniculum</i>	PI-561057	Wild: Barnes Co., ND
<i>Agastache foeniculum</i>	PI-561058	Wild: Cass Co., MN
<i>Agastache foeniculum</i>	PI-561059	Wild: Hennepin Co., MN
<i>Agastache foeniculum</i>	PI-561060	Wild: Morden, Manitoba
<i>Agastache foeniculum</i>	PI-561061	Wild: Las Animas Co., CO
<i>Agastache foeniculum</i>	PI-561062	Wild: Spruce Woods Park, Manitoba
<i>Agastache foeniculum</i>	PI-561063	Wild: Whiteshell Park, Manitoba
<i>Agastache nepetoides</i>	PI-561064	Wild: Iowa
<i>Agastache rugosa</i>	A-8411	Cultivated: Quebec
<i>Agastache rugosa</i>	A-10207	Cultivated: Putaway, Poland

medium was a (1:2:2) mixture of soil, peat, and perlite. The host range of the *Agastache* strain of *Verticillium* was tested on other species of Lamiaceae (Table 2) using the same procedures as described for the accessions of *Agastache*.

Inoculation methods. The lower part of the root system of individual plants was removed from each of the 21 plants comprising an experimental unit. The upper part of the root system remaining attached to the plants was subsequently placed in contact with 210 ml of conidial suspension contained in a polystyrene box (15 cm × 30 cm × 7 cm). After 5 min the plants were removed and returned to the plastic cones which had been partially refilled with autoclaved growing medium.

Experimental design. The 14 *Agastache* accessions, inoculated with each of 3 strains of *Verticillium dahliae* and a sterile water control, were screened for reaction to *Verticillium* using 2 replicates arranged in a complete, randomized block (with 21 plants comprising an experimental unit). The host range of infection of the *Agastache* strain of *Verticillium* within 9 species of Lamiaceae was evaluated in a complete, randomized block design using 2 replicates of 21 plants each per species. A sterile water control was also included in the experiment.

Plants were observed for symptoms of disease or abnormal growth approximately every 4 days. Fungal infection was evaluated 8 weeks after inoculation in the *Agastache* screening and 5 weeks after inoculation in the testing of host range by using 3 pieces of stem from each plant. The stem pieces were plated on sorbose medium, a selective medium for *Verticillium*

TABLE 2. Species of Lamiaceae used to determine the host range of the *Agastache* strain of *Verticillium*.

Species	Accession	Origin
<i>Calamintha clinopodium</i> Benth.	PI-326546	Unknown: Yugoslavia
<i>Mentha piperita</i> L.	PI-557971	Cultivar: 'Black Mitcham' ¹
<i>Monarda fistulosa</i> L.	PI-561068	Wild: Polk Co. IO
<i>Ocimum</i>		
<i>basilicum</i> L.	PI-172998	Cultivated: Van, Turkey
<i>gratissimum</i> L.	PI-414201	Cultivated: Beltsville, MD
<i>kilimandscharicum</i> Guercke	PI-414205	Cultivated: Beltsville, MD
<i>Origanum vulgare</i> L.	PI-383835	Unknown: Yugoslavia
<i>Pycnanthemum pilosum</i> Nutt.	Ames-5021	Cultivated: Ingham Co., MI
<i>Salvia azurea</i> Lam.	PI-421551	Cultivar: 'Nekan' Marion Co., KS

¹Cuttings obtained from the National Clonal Germplasm Repository, Corvallis, Oregon.

(6), and 2 weeks later, the number of plants from which fungal growth was observed was recorded for each treatment. Statistical analysis of the data was conducted with ANOVA and LSD procedures of the SAS software release 6.06 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513).

RESULTS AND DISCUSSION

Disease development and expression in *Agastache* progressed slowly, even as late as 8 weeks after inoculation. At that time, recovery of the fungus from stem pieces was successful only from certain populations of *Agastache* inoculated with the *Agastache* strain of *Verticillium dahliae* (Table 3). The potato and peppermint strains of *Verticillium* were not isolated from stems in any treatments.

Although our inoculations did not produce full expression of disease symptoms, apparent specificity of the strains of *Verticillium dahliae* was noted. The fungus was successfully recovered from *Agastache* only from plants inoculated with the *Agastache* strain. This limited recovery was not

TABLE 3. *Verticillium* infections in upper stems of *Agastache* accessions.

<i>Agastache</i> accession	Species	Infected plants ¹
A-8411	<i>Agastache rugosa</i>	21a ²
A-10207	<i>Agastache rugosa</i>	10 b
PI-561063	<i>Agastache foeniculum</i>	5 bc
PI-561056	<i>Agastache foeniculum</i>	2 bc
PI-561057	<i>Agastache foeniculum</i>	2 bc
PI-561058	<i>Agastache foeniculum</i>	2 bc
PI-561059	<i>Agastache foeniculum</i>	2 bc
PI-561061	<i>Agastache foeniculum</i>	2 bc
PI-561054	<i>Agastache foeniculum</i>	0 c
PI-561055	<i>Agastache foeniculum</i>	0 c
PI-561060	<i>Agastache foeniculum</i>	0 c
PI-561062	<i>Agastache foeniculum</i>	0 c
A-10206	<i>Agastache foeniculum</i>	0 c
PI-561064	<i>Agastache nepetoides</i>	0 c

¹As observed 8 weeks after inoculation via a root-dip in an *Agastache* strain of *Verticillium dahliae*; controls were *Solanum tuberosum* 'Russet Burbank' (0% infection with *Agastache* strain, 100% infection with potato strain), *Mentha piperita* 'Black Mitcham' (0% infection with *Agastache* strain, 100% infection with peppermint strain).

²Means followed by the same letter are not significantly different by LSD. LSD = 7.6%; $\alpha \leq 0.05$.

due to the loss of pathogenicity, as the other 2 strains demonstrated pathogenicity on peppermint (cv. Black Mitcham) and potato (cv. Russet Burbank) plants, respectively, shortly after these strains were used to inoculate *Agastache* (8). In addition, the strain of *Verticillium* isolated from *Agastache* was unable to infect either peppermint or potato.

Clear symptoms of disease, such as leaf curling, plant wilting, and death, developed in individuals of *Pycnanthemum pilosum* and *Salvia azurea* whereas other species of Lamiaceae were infected with the *Agastache* strain of *Verticillium* (Table 4), but no visual symptoms were detected. Widrechner (22) previously reported vascular wilt of unknown etiology occurring in plots of *Salvia azurea*. Possibly, the *Agastache* strain of *Verticillium* is similar in pathogenicity to the *Salvia* strains of *Verticillium* reported by Baker and Locke (2) in California and by Evans and Gleeson (7) in Australia. *Verticillium* was successfully isolated from upper stem pieces of all species inoculated in the test except for *Mentha piperita*, *Monarda fistulosa*, and *Origanum vulgare*. Results from this experiment reinforce the hypothesis that the *Agastache* strain of *Verticillium* has a host range which differs from that of the peppermint strain of *Verticillium*.

Significant differences among the *Agastache* populations tested for reaction to the *Agastache* strain were observed (8). Accessions A-8411 and A-10207, both *Agastache rugosa*, had the highest degrees of *Verticillium*

TABLE 4. *Verticillium* infections in the upper stem of various Lamiaceae species.

Species	Dead plants ¹	Infected plants ¹
	———— (%) ————	
<i>Salvia azurea</i>	33	90a ²
<i>Ocimum gratissimum</i>	0	52 b
<i>Ocimum kilimandscharicum</i>	0	48 b
<i>Ocimum basilicum</i>	0	38 bc
<i>Calamintha clinopodium</i>	0	14 cd
<i>Pycnanthemum pilosum</i>	10	14 cd
<i>Mentha piperita</i>	0	0 d
<i>Monarda fistulosa</i>	0	0 d
<i>Origanum vulgare</i>	0	0 d

¹As observed 5 weeks after inoculation via a root-dip in an *Agastache* strain of *Verticillium*.

²Means followed by the same letter are not significantly different by LSD. LSD = 25.4%; $\alpha \leq 0.05$.

infection (percentage of plants infected) with significant differences noted between *Agastache rugosa* and 5 accessions of *Agastache foeniculum* and the 1 accession of *Agastache nepetoides*. No significant difference in infection between *Agastache foeniculum* and *Agastache nepetoides* was observed. In addition, no significant differences in susceptibility to *Verticillium* among accessions of *Agastache foeniculum* were noted. These latter results undoubtedly occurred because, at most, only 1 of the 21 plants in an experimental unit was infected.

Based on our results and on 3 reports of the susceptibility of *Agastache rugosa* to *Verticillium* (1,3,22), soils infested with *Verticillium* should be avoided when cultivating *Agastache rugosa*. Observations of the reaction of *Agastache foeniculum* and *Agastache nepetoides* to *Verticillium* wilt suggest that these species may be better choices for establishing plantings of *Agastache* for honey bee forage, aromatic-oil production, and other herbal or medicinal uses. Any conclusions regarding the ultimate range of susceptibility in *Agastache nepetoides* and *Agastache rugosa*, however, can only be drawn after a more comprehensive screening. Populations of *Agastache foeniculum* and *Agastache nepetoides* may be valuable sources of resistance to *Verticillium* for the improvement of *Agastache rugosa* if hybrid sterility barriers impeding gene flow among these species (19) can be overcome.

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