Foliar Blight of Bigflower Vetch Caused by *Ascochyta fabae* f. sp. *vicia*

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ABSTRACT

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Bigflower vetch (*Vicia grandiflora* var. *kitaibeliana*) is a winter-annual legume developed for use in pastures and as a winter cover crop in specialized situations. During evaluation in a rotation experiment in Connecticut, bigflower vetch became severely diseased by a foliar blight. Symptoms consisted of reddish brown spots with tan centers on leaves. Spots were oval and often coalesced to form larger blotches. A fungus identified as *Ascochyta fabae* was isolated from diseased tissues, and this fungus induced leaf spots plus necrosis of stems, petioles, and tendrils on bigflower vetch by artificial inoculation. The fungus caused leaf spots (<2 mm in diameter) on *V. faba, V. hirsuta*, and *V. tenuifolia*, minor leaf flecking on four other legume species, and no symptoms on 54 other species. Only bigflower vetch was susceptible. Because of the very narrow host range of the form of *A. fabae* from bigflower vetch, rotation of bigflower vetch with other legumes may not be problematic. This strain of *A. fabae* has the potential, however, to greatly impair the productivity of bigflower vetch in the northeastern United States and possibly elsewhere. *Ascochyta fabae* f. sp. *vicia* is proposed to describe the specialized form of the fungus pathogenic to bigflower vetch.

Bigflower vetch (*Vicia grandiflora* Scop. var. *kitaibeliana* W. Koch 'Woodford') is a self-regenerating winter-annual legume developed by the University of Kentucky (16) and is the only cultivar of this species available. This species may be useful for upgrading predominantly grass pastures, as a crop for wildlife, and as a winter cover crop after agronomic and vegetable crops.

During the third year of a rotational experiment with sweet corn in Connecticut, bigflower vetch developed a severe foliar blight. All aboveground plant tissues were blighted, resulting in leaf and stem death. Pycnidia were evident in some of the dead tissue. This blight was first observed in December on the second-year stand of bigflower vetch, and symptoms became more severe during the following spring. Only a leaf spot caused by *Botrytis* sp., an unidentified rust, Wisconsin pea stunt virus (16), and *Sclerotinia sclerotiorum* (Lib.) deBary (11) were reported previously on bigflower vetch. At least 18 fungal leaf spots of other species of *Vicia* have been reported (5), including those caused by *Ascochyta fabae* Speg., *A. pinodes* Jones, *A. pisi* Lib., *A. vicia* Lib., and an unidentified species of *Ascochyta*. These fungi are similar in morphology and have overlapping host ranges. *A. viciae* is a questionable species, as it has been assigned synonymy with *A. lethalis* (9) and *A. pisi* (1).

This disease on bigflower vetch was of concern not only because it rendered the crop less productive, but also because if the disease developed in other leguminous vegetables, then bigflower vetch might not be suitable for use in rotation systems with such crops.

The objectives of this research were to identify the organism causing the foliar blight of bigflower vetch and to determine its pathogenicity to bigflower vetch and to other legumes that might be used in rotation with bigflower vetch.

MATERIALS AND METHODS

Pathogen isolation and identification. Diseased plants for analysis were collected from experimental plots in Connecticut. Plant tissue was washed in running tap water for 15 min, immersed in 1.25% sodium hypochlorite for 2 min, and rinsed several times in sterile distilled water. Pieces of leaflets, petioles, stems, and tendrils were placed on the surface of 1.5% water agar acidified to pH 2.6. After 48 hr in the dark at 21 ± 1 C, tips of emerging hyphae were transferred to potato-dextrose agar (PDA) at a pH of 5.3. Cultures were maintained at 21 ± 1 C under 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ of cool-white fluorescent light with a 12-hr photoperiod. Cultures were also grown on oatmeal agar (OMA) and autoclaved leaves of bigflower vetch. The fungus was characterized after 14 days on OMA based on spore shape, size, and septation; culture morphology; size of pycnidia; and color of pycnidial ooze (12–14).

Pathogenicity tests. Conidia were washed from 14-day-old cultures grown on V8 juice agar (10) that had been maintained as described above. Concentrations of conidia in an aqueous suspension were adjusted to 25×10^5 ml^{-1}. Tween 20 was added to produce a concentration of 0.25% (v/v). Inoculum was misted until runoff onto 50 12-wk-old bigflower vetch plants that were growing in 500-cm^3 clay pots containing a commercial potting mix. Inoculated plants were maintained at 21 ± 1 C with saturated relative humidity in darkness for 48 hr following inoculation (8), and plant surfaces were allowed to dry slowly before returning the plants to the greenhouse maintained at 20 ± 5 C. Disease symptoms were evaluated 11 days later when symptoms on bigflower vetch were severe. Symptomatic plant tissues were surface-disinfested as described and placed on the surface of acidified PDA (pH 4.2) for reisolation of the pathogen. Tips of hyphae growing from symptomatic tissue were transferred to PDA. Resulting cultures were compared with the original isolate for general appearance and growth rate.

Host range. Because pathogenicity to specific hosts has been used previously to identify species of *Ascochyta*, a broad array of legumes was evaluated in a series of inoculation experiments using the same isolate as in the pathogenicity test. A minimum of 14 12-wk-old plants were used per species. Bigflower vetch was included in each inoculation, and noninoculated plants were maintained as controls. Inoculations were repeated only when symptoms on bigflower vetch were not severe. In reports of pathogenicity of *Ascochyta* spp. on *Lathyrus* and *Vicia*, often the species of these hosts are not given; therefore, as many species of these genera as were available were included in the host-range inoculations. The following legumes were evaluated for disease response: *Arachis hypogea* L.,

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Cajanus cajan (L.) Millsp., *Coronilla varia* L., *Glycine max* (L.) Merr., *Lens culinaris* Medik., *Lespedeza cuneata* (Dum. Cours.) G. Don, *Lotus corniculatus* L., *Medicago lupulina* L., *M. sativa* L., *Phaseolus lunatus* L. (three cultivars), *P. vulgaris* L. var. *humilis* Alef. (two cultivars), *Pisum sativum* L., *Trifolium alexandrinum* L., *T. ambiguum* Bieb., *T. hybridum* L., *T. incarnatum* L., *T. pratense* L., *T. repens* L. (two cultivars), and *Vigna unguiculata* (L.) Walp. Species of *Vicia* inoculated included: *articulata* Hornem, *cracca* L., *ervilia* L. Willd., *faba* L., *fulgens* L., *hirsuta* (L.) S.F. Gray, *hybrida* L., *hycanica* L., *lathyroides* L., *lutea* L., *monantha* Retz., *narbonensis* L., *pannonica* Crantz, *pisiformis* L., *sativa* L., *tenuifolia* Roth, and *tetrasperma* (L.) Moench. Species of *Lathyrus* inoculated included: *annuus* L., *aphaca* L., *articulatus* L., *chloranthus* L., *cicera* L., *clymenum* L., *decaphyllus* L., *erectus* L., *gorgoni* Parl., *hierosolymitanus* L., *hirsutus* L., *inconspicuus* L., *japonicus* (Roth) Willd., *latifolius* L., *ochrus* (L.) de Candolle, *pseudo-cicera* L., *roseus* Steven, *sativus* L., *sinensis* (Torn.) Savi, *sylvestris* L., *szowitzii* L., and *tingitanus* L.

RESULTS AND DISCUSSION

Pathogen isolation and identification. Only one fungus was consistently isolated from tissues of bigflower vetch. This fungus produced an ashen gray, floccose mycelium that covered the central half of the colony on PDA. Brown pycnidia with papillate ostioles were abundant, and these contained hyaline conidia that were straight or slightly curved. Most conidia had one septum, although aseptate and two- and three-septate spores also were present. Conidia produced on PDA ranged from 4.3–5.7 μm wide × 12.1–16.2 μm long (150 spores measured). More three- and four-celled conidia were produced on autoclaved bigflower vetch leaves than on artificial media, although the frequency of their occurrence on OMA could be as high as 25%. Generally, conidia were not constricted at the septa, and, en masse, the pycnidial ooze was tan or buff. All gross morphological characteristics of colonies, pycnidia, and conidia placed this fungus in the genus *Ascochyta* (15).

Conidia of the *Ascochyta* sp. from bigflower vetch were larger, especially in width, than those reported for *A. pinodes* (3–4.5 × 8–16 μm [13]) or *A. pisi* (3–4.5 × 10–16 μm [12]) and most closely resembled those of *A. fabae* (4.2–5.3 × 15.3–17.8 μm [7]). The pycnidial ooze of the bigflower vetch isolate was tan or buff as described also for *A. pinodes* (13) and *A. fabae* (4) but different from the bright red or pink of *A. pisi* (12). Conidia of *A. pinodes* (13) and *A. pisi* (12) are constricted at the septa, which is different from those of *A. fabae* (2) and those of the *Ascochyta* sp. from bigflower vetch. Pycnidia of *A. pinodes* (13) and *A. pisi* (12) are reported to range in diameter from 100 to 200 μm, and those of *A. fabae* (14), from 200 to 250 μm. Pycnidia of the *Ascochyta* sp. from bigflower vetch growing on OMA ranged from 147 to 297 μm (50 pycnidia measured). On the basis of characteristics of conidia, pycnidia, and pycnidial ooze, the isolate of *Ascochyta* from bigflower vetch most closely resembled *A. fabae*.

Pathogenicity tests. Inoculation of bigflower vetch plants with *A. fabae* caused symptoms that were indistinguishable from those that occurred naturally in the field. Dark, reddish brown spots with tan centers formed on leaflets (Fig. 1). These ranged in size from 3 mm in diameter to the entire surface of the leaflet. Spots were oval and somewhat irregular and often coalesced to produce larger blotches. Petioles, stems, and tendrils had necrotic areas, and some were killed (Figs. 1 and 2). Severe disease caused death of plant tissues and a general blighted appearance. The fungus was reisolated from several different types of plant tissue and did not differ in culture from the original field isolate.

Host range. Bigflower vetch was severely diseased in all tests. Black leaf spots less than 2 mm in diameter occurred on leaves of *V. faba, V. hirsuta*, and *V. tenuifolia*, and minute flecks occurred on *L. roseus*. No symptoms were observed on any of the other legumes tested. Noninoculated control plants remained symptomless throughout all inoculation periods. The fungus from bigflower vetch exhibited a very limited host range.

Speciation in *Ascochyta* is very difficult because discrete morphological characters have not been described. Over 100 species are listed on hosts in the United States (5), and 328 species have been recognized in what was formerly the USSR (9).

The literature on the host range of *Ascochyta* species lacks complete agreement but in general supports the concept that *A. pinodes* and *A. pisi* attack plants in several genera (5,15), whereas *A. fabae* has a very limited host range (3,14). The strain of *A. fabae* that attacks lentils (6) appears to be as restricted to that host as our isolate is to bigflower vetch.

In the past, host specificity has been used to speciate fungi. Now, however, important physiologic differences are usually designated as form species, which are not included in the International Code of Botanical Nomenclature. Gossen et al (6) described a strain of *A. fabae* that was pathogenic only to lentil and, in order to designate the uniqueness of that strain, called it form species, *lentis*. We agree with this approach. Therefore, on the basis of the morphological characters available and the host range comparison, we advocate that the fungus from bigflower vetch be designated *A. fabae* Speg. f. sp. *vicia* Leath, DeGregorio, & Ashley.

The narrow host range of *A. f. vicia* suggests that the use of bigflower vetch in rotation with other legumes would not jeopardize the other legumes. *A. f. vicia* does, however, pose a serious threat to the performance of bigflower vetch in the

Fig. 1. Symptoms of infection by *Ascochyta fabae* f. sp. *vicia* after artificial inoculation of foliage of bigflower vetch.

Fig. 2. Necrosis on stems of bigflower vetch caused by artificial inoculation with *Ascochyta fabae* f. sp. *vicia*.

northeastern United States and possibly other regions as well.

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