Fungi Resident in Chickpea Debris and their Suppression of Growth and Reproduction of *Didymella rabiei* under Laboratory Conditions

F. M. Dugan¹, S. L. Lupien¹, M. Hernandez-Bello², T. L. Peever³ and W. Chen¹

Authors’ addresses: ¹USDA-ARS, Washington State University, Pullman, WA 99164, USA; ²Department of Plant Pathology, University of California, Davis, CA 95616, USA; ³Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA (correspondence to F. M. Dugan. E-mail: fdugan@mail.wsu.edu)

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Abstract

Fungi colonizing senescent chickpea (*Cicer arietinum*) stems and postharvest debris from Pullman, WA, were enumerated and identified with the objective of finding species potentially useful for biological control of *Didymella rabiei* (conidial state = *Ascochyta rabiei*), causal agent of Ascochyta blight. In addition to *D. rabiei*, primary colonizers were, in order of decreasing abundance, *Alternaria tenuissima*, *Al. infectoria*, *Ulocladium consortiale*, *Epicoccum purpurascens*, *U. atrum* and *Fusarium pseudograminearum*. Present at lower frequencies were *Al. malorum*, *Cladosporium herbarum*, *Aureobasidium pullulans*, *Clonostachys rosea* and miscellaneous anamorphic ascomycetes. On agar media and autoclaved chickpea stems, *Au. pullulans* consistently grew faster than *As. rabiei*, and excluded *As. rabiei* from the substrate. When stems received prior inoculation with *Au. pullulans* or *Cl. rosea*, followed by inoculation with compatible mating types of *D. rabiei*, formation of pseudothecia and pycnidia of *D. rabiei* was suppressed. Results suggest that *Au. pullulans* and *Cl. rosea* can inhibit *As. rabiei* and its sexual stage, *D. rabiei*, on chickpea debris. *Clonostachys rosea* formed appressoria on, then invaded, hyphae of *D. rabiei*. Small-scale field experiments using *Au. pullulans* and *Cl. rosea* have been initiated.

Introduction

The adoption of reduced tillage practices to control soil erosion in US Pacific Northwest (PNW) cropping systems has created conditions that are ideal for the survival and reproduction of *Ascochyta rabiei* (Pass.) Lab., the causal agent of Ascochyta blight of chickpea (*Cicer arietinum* L.). The sexual stage, *Didymella rabiei* (Kovachevski) Arx, develops during the winter on chickpea debris, and ascospores infect newly emerging chickpeas the following season (Kaiser, 1992). One of the best control measures for Ascochyta blight is the deep ploughing of infected plant residues (Kaiser and Hannan, 1987); however, this practice is incompatible with soil conservation practices. The increasing amount of plant debris left on the soil surface is thought to have led to a large increase in inoculum for Ascochyta blight epidemics and to the increased severity of blight epidemics observed in the PNW the past few years. Given the importance of soil erosion control and the inevitable move towards reduced tillage in PNW agriculture, control of Ascochyta blight will increasingly depend on suppressing the sexual stage of the pathogen.

The primary type of inoculum for Ascochyta blight epidemics in the PNW is thought to be ascospores (Trapero-Casas and Kaiser, 1992a,b; Trapero-Casas et al., 1996). This is based both on low rates of seed infection of PNW seed lots, and anecdotal observations of disease pattern and timing in the field (T. L. Peever, W. Chen and W. J. Kaiser, unpublished data) as well as on indirect evidence from mating type ratios and multilocus genetic disequilibrium tests with genetic markers which indicate a recombined population structure (Peever et al., 2004). Ascospores land on chickpea plants, infect directly and form lesions, which produce pycnidia. Secondary spread of Ascochyta blight within chickpea fields during the growing season is dependent upon rainfall events, which disperse conidia to uninfected plants and create appropriate environmental conditions for infection (Trapero-Casas and Kaiser, 1992a,b). The sexual stage is formed on chickpea debris colonized by the fungus the previous season and requires approximately 2 months at cool temperatures (5–10°C) to mature and produce ascospores. Ascospores are released under specific environmental conditions, are wind-borne, and can infect chickpea plants several hundred metres or more from the source, while conidia are splash-dispersed only short distances (Kaiser, 1992; Trapero-Casas et al., 1996). Long-distance movement of ascospores is the
most likely route by which the pathogen is moved among chickpea fields and from infected to uninfection fields. Our objectives here were to identify the primary fungal competitors of *D. rabiei* in colonization of chickpea debris, and to conduct preliminary tests with one or more of these competitors to assess the ability of these fungi to inhibit growth and reproduction of *D. rabiei*.

**Materials and Methods**

**Floristic analysis of fungi colonizing chickpea debris**

Chickpea stems were collected on 27 August 2003 at Washington State University’s Spillman Research Farm, Pullman, Washington and 9 September 2004 at a commercial farm near Genesee, Idaho. Stems from the Washington site were collected at harvest; those from Idaho approximately 2 weeks after harvest. For each location, segments 2 cm long were excised from 100 stems. Prior to transfer to standard culture dishes containing agar media, 25 of the segments were washed in running tap water for 2 h, 25 were disinfested by immersion for 10 s in 70% ethanol then rinsed with sterile water, and 50 were disinfested by immersion for 1 min in 0.5% NaOCl then rinsed with sterile water. Approximately one-third of the stem segments from each of the two locations and from each of the disinfection treatments were plated on malt agar amended with rose bengal (Dugan and Lupien, 2002) and approximately two-thirds were plated to half-strength *V8* agar (1/2V8; Stevens, 1981). Each of the agar media was amended with 50 μg/ml each of streptomycin sulphate and tetracycline hydrochloride (Sigma®, St Louis, MO, USA). Cultures were incubated under ambient laboratory conditions (approximately 22°C, daytime fluorescent light), during which time fungal isolates were subcultured to slants of 1/2V8 and incubated under near ultraviolet and fluorescent light (12 h/12 h light–dark cycle) at ambient temperature for recovery of fungi. Isolates recovered were identified by published morphological and physiological criteria (Simmons, 1967, 1986, 1990, 1995; Ellis, 1971, 1976; Kushwaha and Agrawal, 1976; Hermanides-Nijhof, 1977; Nelson et al., 1983; dos Santos et al., 1993; de Hoog and Yurlova, 1994; Andersen and Thrane, 1996; Seifert, 1996; Aoki and O’Donnell, 1999; Ho et al., 1999; Yurlova et al., 1999; Schroers, 2001). Taxa which were recovered in highest abundance (*Alternaria, Ulocladium*), were subsampled at random by generation of single-spore isolates for every third isolate (for *Ulocladium*) to sixth isolate (for *Alternaria*).

**Relative growth rates and interspecies growth inhibition**

*Didymella rabiei*, *Aureobasidium pullulans* and *Clonostachys rosea* Strains of *D. rabiei*, *Au. pullulans* and *Cl. rosea* isolated from chickpea are listed in Table 1.

**Relative growth on chickpea stems**

For assessing relative growth of *As. rabiei* (isolates AR19, AR628) and *Au. pullulans* (AuP12, AuP26), incubation chambers were constructed by fixing autoclaved segments of chickpea stems to glass rods with drops of silicone adhesive, and placing the rods and stems on a stainless steel disc over a pool of sterile distilled water (higher humidity) or a mixture of 80% sterile distilled water and 20% glycerol (lower humidity) within a 15-cm-diameter glass culture dish (Fig. 1). Within each chamber, four groups of two stems each were inoculated at stem midpoint with a single isolate of one species or the other, and growth rates were measured and averaged for each stem so that average growth rates were twice obtained for two isolates of *As. rabiei* and two isolates of *Au. pullulans* within a single chamber. There were two chambers for each level of relative humidity (RH) and the design was blocked by chamber for statistical analysis. Chambers were sealed with Parafilm® (Menasha, WI, USA) and incubated under fluorescent and near ultraviolet light (12 h light/12 h dark) at 15°C. Relative humidity was monitored by construction of two additional chambers, each enclosing a Humidity-On-A-Card™ (Houston, TX, USA). The entire experiment was repeated at 20°C. An analogous set of chambers using *As. rabiei* (AR19, A628) and *Cl. rosea* (CP63E, CP98B) was constructed and incubated at 20°C with RH ≥90%. In all instances, stem ends were excised from stems at completion of the experiment, and transferred to 1/2V8 for confirmation of identity of the fungi and to check for contamination.
**Interspecies growth inhibition of As. rabiei and Au. pullulans on chickpea stems**

Another set of chambers (Fig. 2) was produced and inoculated on the same date as the experiment above. Within a single chamber, four groups of two stems each were inoculated at stem midpoint with *As. rabiei* (either AR19 or AR628), and at both stem ends with *Au. pullulans* (either AuP12 or AuP26), such that, within a single chamber, each AR isolate was paired once with each AuP isolate, and the colonies were allowed to grow towards one another. There were two replicate chambers for each combination of temperature (15°C/17°C or 20°C) and RH (RH ≥ 80% or RH ≥ 90%). Colony growth rate and other characters were monitored as colonies converged. Reactions (colour, presence of aerial hyphae and/or fruiting bodies) were noted every 2 days for each colony on each stem at 10–60×. About 2–4 days after the slowest growing isolate of *As. rabiei* had reached ends of the stems above (Fig. 1), approximately 5 mm from ends of the stems in the chambers designed to test inhibition (Fig. 2) were harvested and placed onto 1/2V8 and incubated at laboratory conditions as above for any recovery of *As. rabiei* from the ends occupied by *Au. pullulans* colonies. The assumption was that the stems inoculated only at midpoint with *As. rabiei* (Fig. 1) would give a measure of the time necessary for uninhibited *As. rabiei* to grow to the ends of the stems, so that subsequent failure to recover *As. rabiei* from the distal ends of corresponding stems illustrated in Fig. 2 would constitute further evidence of the ability of *Au. pullulans* to exclude *As. rabiei* from the substrate. Data from each experiment were subjected to **proc anova** and/or **proc glm** in **sas 8.2** (SAS Institute, Inc., Cary, NC, USA) and/or **anova** in **systat 9.0** (SPSS Science, Chicago, IL, USA).

**Inhibition of formation of ascospores and conidia by preinoculation of substrate with *Au. pullulans***

Stem sections (approximately 3.5 cm each) were excised from healthy dried chickpea plants, autoclaved twice on each of 2 successive days for 30 min at 121°C while suspended over 2.5 cm of water in glass beakers. Multiple pairs of isolates of *As. rabiei* of compatible mating types were employed to initiate crosses on stems that were preinoculated with *Au. pullulans*. Sets of controls were: (i) stems not preinoculated with *Au. pullulans* and inoculated with compatible mating types of *As. rabiei*, (ii) two self-crosses as negative controls, and (iii) two self-crosses of each mating type as positive control.
controls for comparison with outcrosses. *Didymella rabiei* is heterothallic with a bipolar mating system (Wilson and Kaiser, 1995; Barve et al., 2003). Crosses are shown in Table 2.

**Preinoculation** Inoculum of *Au. pullulans* isolate AuP12 was increased by streaking onto V8 medium and incubating at 22–25°C under combined cool white and near UV light on an alternating 12-h photoperiod for 6 days. Conidia were harvested by washing the agar surface with sterile deionized water and concentration was adjusted to $6.25 \times 10^6$ conidia/ml. Autoclaved chickpea stems were immersed in conidial suspension for 2.5 h with intermittent mild agitation. Stems were transferred to chambers (sterile glass Petri dishes with 15 layers of sterile Whatman No. 1 filter paper with 10 ml sterile deionized water) and incubated until colonies made contact, at which time they were examined for reaction zones. The experiment was replicated.

**Results**

**Floristic analysis** Fungi recovered from chickpea stems and debris in 2003 and 2004 are listed in Table 3. In order of relative abundance, the most frequently isolated fungi were *Al. tenuissima, Al. infectoria, U. consortiale, Epicoccum* sp. 1, 2003a 2004b.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Al. tenuissima</em></td>
<td>36 35</td>
</tr>
<tr>
<td><em>Al. infectoria</em></td>
<td>12 20</td>
</tr>
<tr>
<td><em>Al. madorum</em></td>
<td>4 0</td>
</tr>
<tr>
<td><em>Ul. consortiale</em></td>
<td>12 6</td>
</tr>
<tr>
<td><em>Ul. atrum</em></td>
<td>2 6</td>
</tr>
<tr>
<td><em>Ep. purpurascens</em></td>
<td>6 4</td>
</tr>
<tr>
<td><em>Fusarium pseudograminearum</em></td>
<td>5 2</td>
</tr>
<tr>
<td><em>Fusarium acuminatum</em></td>
<td>0 3</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>1 4</td>
</tr>
<tr>
<td><em>Fusarium spp.</em></td>
<td>1 2</td>
</tr>
<tr>
<td>Unidentified</td>
<td>6 3</td>
</tr>
<tr>
<td><em>Clad. herbarum</em> (Pers.:Fr.) Link</td>
<td>3 2</td>
</tr>
<tr>
<td><em>Clad. cladosporioides</em> (Fresen.) G. de Vries</td>
<td>0 2</td>
</tr>
<tr>
<td><em>Cladosporium sp.</em></td>
<td>1 &lt;1</td>
</tr>
<tr>
<td><em>Phomopsis spp.</em></td>
<td>3 4</td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em> (de Bary) G. Arnauld</td>
<td>2 3</td>
</tr>
<tr>
<td><em>Clonostachys rosea</em> (Link:Fr.) Schroers et al.</td>
<td>2 0</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> Pers.:Fr.</td>
<td>0 1</td>
</tr>
<tr>
<td><em>Al. sp.</em></td>
<td>&lt;1 &lt;1</td>
</tr>
<tr>
<td><em>Asc. sp.</em></td>
<td>0 &lt;1</td>
</tr>
<tr>
<td><em>Botrytis</em> keratinophilum Kushwaha &amp; S.C. Agarwal</td>
<td>&lt;1 0</td>
</tr>
<tr>
<td><em>Curvularia inaequalis</em> (Shear) Boedijn</td>
<td>&lt;1 0</td>
</tr>
<tr>
<td><em>Chaetomium</em> sp.</td>
<td>0 &lt;1</td>
</tr>
<tr>
<td><em>Clad. macrocarpum</em> G. Preuss</td>
<td>0 &lt;1</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> Schlechtend.:Fr.</td>
<td>0 &lt;1</td>
</tr>
<tr>
<td><em>Humicola</em> sp.</td>
<td>&lt;1 &lt;1</td>
</tr>
<tr>
<td><em>Mucor</em> sp.</td>
<td>0 &lt;1</td>
</tr>
<tr>
<td><em>Pithomyces chartarum</em> (Berk. &amp; M.A. Curtis) M.B. Ellis</td>
<td>&lt;1 0</td>
</tr>
</tbody>
</table>

Table 2: Most abundant fungal colonists of chickpea stems

<table>
<thead>
<tr>
<th>Nine outcrosses</th>
<th>Total percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR20e (MAT1-2) × AR630 (MAT1-1)</td>
<td>36 35</td>
</tr>
<tr>
<td>AR20e (MAT1-2) × AR21d (MAT1-1)</td>
<td>12 20</td>
</tr>
<tr>
<td>AR20e (MAT1-2) × AR462 (MAT1-1)</td>
<td>4 0</td>
</tr>
<tr>
<td>AR460 (MAT1-2) × AR630 (MAT1-1)</td>
<td>12 6</td>
</tr>
<tr>
<td>AR460 (MAT1-2) × AR21d (MAT1-1)</td>
<td>2 6</td>
</tr>
<tr>
<td>AR460 (MAT1-2) × AR462 (MAT1-1)</td>
<td>6 4</td>
</tr>
<tr>
<td>AR428 (MAT1-2) × AR630 (MAT1-1)</td>
<td>5 2</td>
</tr>
<tr>
<td>AR428 (MAT1-2) × AR21d (MAT1-1)</td>
<td>0 3</td>
</tr>
<tr>
<td>AR428 (MAT1-2) × AR462 (MAT1-1)</td>
<td>1 4</td>
</tr>
</tbody>
</table>

Two self-crosses AR20e × AR20e | 1 2 |
| AR21d × AR21d | 6 3 |

a265 isolates from chickpea stems 2 weeks postharvest.

b358 isolates from chickpea stems at harvest.

*Non-sporulating, becoming non-viable, or otherwise unidentified.
stem ends inoculated with *Aureobasidium pullulans* 20°C. Both species slowed dramatically and sometimes ceased growing on the stems. Colonies did not merge and a reaction zone was apparent (Fig. 2). In every chamber,mainly contacting the colony of the other species. Colonies did not intermingle. In every chamber, main-

## Table 4

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth (species)</th>
<th>Growth (strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15°C</td>
<td>20°C</td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>18.63(^{a}) 28.42(^{a})</td>
<td>AuP26 18.75(^{ab}) 35.28(^{ab})</td>
</tr>
<tr>
<td><em>Ascochyta rabiei</em></td>
<td>14.19(^{a}) 16.48(^{ab})</td>
<td>AuP12 18.50(^{b}) 21.56(^{b})</td>
</tr>
<tr>
<td></td>
<td>8.38(^{a}) 22.20(^{a})</td>
<td>AuP6 8.75(^{a}) 25.25(^{a})</td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>6.50(^{b}) 12.28(^{ab})</td>
<td>AR19 14.13(^{ab}) 15.60(^{ab})</td>
</tr>
<tr>
<td><em>Ascochyta rabiei</em></td>
<td>5.63(^{ab}) 11.74(^{ab})</td>
<td>AR19 5.63(^{ab}) 11.74(^{ab})</td>
</tr>
</tbody>
</table>

*Relative humidity (RH) high, 290% RH; RH low, 280% RH. Within each RH level, means with the same upper case letter do not differ at P ≤ 0.05 (LSD). Within each RH level, means with the same lower case letter do not differ at P ≤ 0.10 (LSD; N = 32).*

purpurascens and *U. atrum*. Also notable were other dematiaceous hyphomycetes including *Aureobasidium pullulans* and *Fusarium* species. *Clonostachys rosea* was isolated only in 2003.

**Relative growth rates and interspecies growth inhibition**

Relative growth on chickpea stems Growth at 15°C was less than at 20°C for both *Aureobasidium pullulans* and *Ascochyta rabiei*, and growth at lower humidity (RH ≥ 80%) was less than at higher humidity (RH ≥ 90%; Table 4). When data were analysed by species, *Aureobasidium pullulans* always grew more quickly than *Ascochyta rabiei*. When analysed by strain, both *Aureobasidium pullulans* strains grew more quickly than the *Ascochyta rabiei* strains, but in some instances differences were not significant (Table 4). When *Cl. rosea* was paired with *Ascochyta rabiei* (at 20°C and RH ≥ 90%) mean growth rates did not differ at P ≤ 0.10 on the basis of species assignment. With regard to strain, the fastest mean growth (20.03 mm) was from *Cl. rosea* CP63E and the slowest (13.88 mm) was from *Cl. rosea* CP98B, with the two *Ascochyta rabiei* isolates being intermediate (data not shown).

Interspecies growth inhibition by *Ascochyta rabiei* and *Aureobasidium pullulans* on chickpea stems At 20°C and RH ≥ 90%, and at 15°C and RH ≥ 90%, *Ascochyta rabiei* and *Aureobasidium pullulans* colonies on the stems grew steadily until contacting the colony of the other species. Colonies did not merge and a reaction zone was apparent (Fig. 2). The distinctive fruiting bodies of each fungus were never observed to intermingle. In every chamber maintained at the lower humidity (RH ≥ 80%), growth slowed dramatically and sometimes ceased well prior to contacting the colony of the other species. In stems inoculated in the centre with a single strain of either species and not inoculated distally (Fig. 1), growth of both species slowed dramatically and sometimes ceased prior to reaching the ends of the stem in chambers with RH ≥ 80%. In each case from the experiments at 20°C + RH ≥ 90% and 15°C + RH ≥ 90%, when stem ends inoculated with *Aureobasidium pullulans* were excised and plated to agar, only *Aureobasidium pullulans* grew from the stem ends. On the controls (excised ends of stems inoculated in the centre with a single fungus), stems inoculated with *Ascochyta rabiei* produced only *Ascochyta rabiei* colonies, and stems inoculated with *Aureobasidium pullulans* produced only *Aureobasidium pullulans* colonies. Prior colonization of the distal portion of the stem by *Aureobasidium pullulans* effectively excluded growth of *Ascochyta rabiei* originating from the stem centre (Fig. 2), at least for the time frame during which the experiment was conducted. In one chamber at 20°C + RH ≥ 90%, one stem of the controls, supposedly inoculated with *Ascochyta rabiei*, was discarded because of contamination or erroneous inoculation with *Aureobasidium pullulans*.

The faster growth of *Aureobasidium pullulans* relative to that of *Ascochyta rabiei* was analogously documented on two agar media, one made with MEA and the other with pulverized chickpea seeds, pods and stems. At both 15 and 20°C strains of *Aureobasidium pullulans* grew more rapidly on MEA than strains of *Ascochyta rabiei* (P < 0.001, data not shown). On the chickpea medium, we also compared growth rates of *Ascochyta rabiei* with rates of *A. infectoria*, *Al. tenuissima*, *Stemphylium*) sp., *U. consortiale*, *E. purpurascens* and *Cl. rosea*, all of which grew faster than *Ascochyta rabiei* (P < 0.01, data not shown). When *Ascochyta rabiei* was paired with other species, inhibition zones were apparent in all crosses, except those involving *Cl. rosea*, in which cases *Cl. rosea* overgrew colonies of *Ascochyta rabiei*.

**Inhibition by *Aureobasidium pullulans* of ascospores and conidia production in *D. rabiei***

The following crosses produced ascospores from four of four attempted crosses in the positive controls: AR630 × AR20e, AR462 × AR20e, AR630 × AR460, AR462 × AR460. The crosses AR462 × AR428 and AR421d × AR460 yielded ascospores from three of four and one of four crosses respectively. The crosses AR21d × AR460, AR630 × AR428 and AR21d × AR428 did not produce ascospores in the positive controls, and were not further used in the analysis. Neither self-cross (AR20e, AR21d) produced ascospores, no ascospores were produced on any stems receiving prior treatment with *Aureobasidium pullulans*, nor were pycnidia seen to mature on any such stems. Hence, the suppression of sexual reproduction of *D. rabiei* by prior inoculation with *Aureobasidium pullulans* is regarded as total amongst all *D. rabiei* crosses producing ascospores in the positive controls, and the suppression of asexual reproduction of *D. rabiei* is similarly regarded as total in all instances in which stems were preinoculated with *Aureobasidium pullulans*. The relative abundance of ascospores produced in the positive controls varied. In most instances, more than 100 ascospores were produced on a given agar plate, but one cross of AR462 × AR20e, one of AR630 × AR460 and one of AR21d × AR460 produced fewer than 50 ascospores.

*Clonostachys rosea* was also effective for inhibiting reproduction of *D. rabiei*. In the positive controls, five of the nine attempted crosses produced ascospores...
inoculation with Cl. rosea of ascospores were not quantified beyond /C212 vs. /C212 and AR460 · (Figs 3 and 4). Interactions of Cl. rosea penetrated hyphae of AR19 and grew internally did stems receiving prior inoculation with Cl. rosea produce mature pycnidia. Prior inoculation with Cl. rosea, as with Au. pullulans, completely suppressed both sexual and asexual reproduction by D. rabiei.

Mycoparasitism of D. rabiei and other fungi by Cl. rosea Both CP63E and CP98B (Cl. rosea) formed appressoria on hyphae of AR19 D. rabiei, and subsequently penetrated hyphae of AR19 and grew internally (Figs 3 and 4). Interactions of Cl. rosea with non-target fungi varied. Aureobasidium pullulans hyphae

tended to grow deeper into the medium while Cl. rosea occupied the agar surface, with hyphae of Cl. rosea eventually contacting hyphae of Au. pullulans and branching prior to prolonged contact. Hyphae of Cl. rosea formed profuse coils and appressoria on hyphae of Al. infectoria, Al. tenuissima, Stemphylium sp. and U. consortiale. Interactions with E. purpurascens were similar to those with Au. pullulans. Penetration of hyphae of the other fungus by Cl. rosea was observed only when Cl. rosea was paired with D. rabiei.

When paired on MEA plates, colonies of D. rabiei were in all instances overgrown by the strains of Cl. rosea, which sporulated on them. There was no evidence of a reaction zone between the two taxa. When paired with other taxa (Au. pullulans and the last five species in Table 1) on MEA, Cl. rosea was uninhibited. Colonies of Cl. rosea overgrew, and to a limited extent sporulated on, the surface of colonies of the taxa with which they were paired. No reaction zones were apparent between Cl. rosea and the other taxa, although in the areas of overlap between Cl. rosea and the colonies of other taxa, Cl. rosea sporulated less prolifically than normal.

Discussion The most frequently recovered fungal colonists of chickpea debris were common dematiaceous hyp-homycetes, most of which have been reported in analogous surveys from other plant substrata (e.g. Dugan and Roberts, 1994; Dugan and Lupien, 2002; Dugan et al., 2002). Conspicuous by its absence in the 2003 survey was Al. alternata (sensu Simmons, 1990, 1995), although Al. tenuissima and Al. infectoria, two common species, were readily recovered, as was the less widely reported Al. malorum, recently reassigned to that genus from Cladosporium (Braun et al., 2003). Also conspicuously absent in 2003–2004 were representatives of Stemphylium (teleomorphs in Pleospora). Both Stemphylium sp. and Pleospora sp. had been encountered in prior inspections of chickpea residue (W. Kaiser and T. Peever, unpublished data), as was Au. pullulans (F. Dugan, unpublished data). We account for the repeated isolation of F. pseudograminearum (F. graminearum group 1) and F. acuminatum by the prevalence of cereals and grasses in the immediate vicinity of our plots and in rotations with chickpea, by occasional grassy weeds, by the persistence of F. graminearum in residues (Pereya et al., 2004) and by the wide range of plant substrata from which these species have been documented (Ginns, 1986; Farr et al., 1989). Fusarium pseudograminearum is commonly isolated from stems of wheat and barley in eastern Washington (T. Paulitz, personal communication).

Amongst those taxa recovered by us from chickpea debris and previously documented as experimental biological control agents against phytopathogens are Au. pullulans, E. purpurascens and U. atrum (e.g. Wittig et al., 1997; Kessel et al., 1999; Castoria et al., 2001). Clonostachys rosea has also been used in numer-
Fungi in Chickpea Debris

ous experiments in biological control (Xue, 2003a,b), including work on chickpea (Burgess et al., 1997; Prasad and Rangeshwaran, 1999). *Clonostachys* species other than *Cl. rosea* have also evoked interest for their potential in biological control (Evans et al., 2003).

Many fungi have been employed as biocontrol agents against phytopathogenic fungi (Dugan, 1996), including phytopathogens that are relatives of *As. rabiei* (Pfender, 1988; Pfender et al., 1993; Ouimet et al., 1997; Philion et al., 1997; Carisse et al., 2000; Bujold et al., 2001; Carisse and Bernier, 2002). We isolated and identified to species none of the fungi utilized and proven effective by these other authors against relatives of *As. rabiei*, with the significant exception of *E. purpurascens*, but we note that isolates denoted as *Aureobasidium* sp. were sometimes utilized by these other workers (Ouimet et al., 1997; Philion et al., 1997).

Candidate organisms for biological control of phytopathogenic fungi should meet the following criteria: (i) well established in nature in the geographical area in which they are to be deployed, (ii) easy to grow in mass fermentation culture, (iii) survive well on organic debris, (iv) non-pathogenic to plants, humans and animals (i.e. should be Biosafety Level 1), (v) non-allergenic to humans and animals and (vi) demonstrated ability to antagonize pertinent pathogens (Dugan, 1996; Goettel et al., 2001). *Aureobasidium pullulans* conforms well to the above criteria (Solomon and Kelks, 1997; Andrews et al., 2002), is well represented in the microflora of chickpea debris, has been the subject of extensive use in other experimental biological control (e.g. Leibinger et al., 1997; Lima et al., 1997; Dik and Elad, 1999; Dik et al., 1999; Schena et al., 1999, 2002; Ippolito et al., 2000; Castoria et al., 2001), and also produces pullulan, a natural adhesive that is used as a food additive (Ducrey et al., 1992) and which helps retain spores on surfaces (Bardage and Bjurman, 1996).

Potential allergenicity is a prime safety concern for fungal biological control agents (Butt and Copping, 2000). *Alternaria*, *Ulocladium* and *Sphomphylum* species produce the Alt-1 allergen (and other allergens as well) and impact large sectors of the human population (Agarwal et al., 1982; Lelong et al., 1986; Horner et al., 1995; Day and Ellis, 2001). *Epococcum purpurascens* and *Cladosporium* species are also important allergens for major sectors of the population (Day and Ellis, 2001; Bish et al., 2004). Approximately 20% of the total human population are atopic and readily sensitized to spore concentrations of 10^3/m^3 in the ambient air (Lacey, 1981). *Aureobasidium pullulans* is less allergenic than the above species, affecting approximately 8–12% of the human population experiencing fungal allergy (Solomon and Kelks, 1997). Like virtually all common fungi that are Biosafety Level 1, *Au. pullulans* has occasionally been isolated from puncture wounds or from immunocompromised patients in clinical settings (de Hoog et al., 2000). We failed to locate any literature implicating *Clonostachys* with allergenic or pathogenic activity in humans or animals. We realize that massive release of mycoparasitic *Cl. rosea* into the environment may have implications for non-target fungi, hence inclusion of the latter into our experiments. Unlike Xue (2003a) we observed no penetration of hyphae of *Alternaria*, but we did observe appressoria of *Cl. rosea* in these interactions. In other respects, our results were similar to those of Xue (2003a), i.e. *Cl. rosea* coiled around hyphae of other fungi.

Based on the above criteria, and considering the ability of our strains of *Au. pullulans* to grow faster than *D. rabiei*, to exclude it from substrate already occupied by *Au. pullulans*, and to completely suppress sexual and asexual reproduction under our experimental conditions, we believe that *Au. pullulans* has potential as a biological control agent against Ascochyta blight of chickpea. Based on its abilities as a mycoparasite, *Cl. rosea* may also be a prospective agent because it is mycoparasitic on *D. rabiei*, successfully suppressed sexual and asexual reproduction in our laboratory, and is neither known for pathogenicity nor allergenicity to humans or animals. We have initiated small-scale field trials using *Au. pullulans* and *Cl. rosea* on chickpea debris infested with *D. rabiei* to determine if the sexual stage can be suppressed by these agents under natural conditions.

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