North American Fungi

Volume 7, Number 6, Pages 1-11
Published May 17, 2012

Clonostachys rhizophaga and other fungi from chickpea debris in the Palouse region of the Pacific Northwest, USA

F.M. Dugan[1], S.L. Lupien[1] and W. Chen[2]

[1]USDA-ARS Western Regional Plant Introduction, [2]USDA-ARS Grain Legume Genetics and Physiology, Washington State University, Pullman, WA 99164

Dugan, F. M., S. L. Lupien, and W. Chen. 2012. *Clonostachys rhizophaga* and other fungi from chickpea debris in the Palouse region of the Pacific Northwest, USA. *North American Fungi* 7(6): 1-11.
doi: http://dx.doi: 10.2509/naf2012.007.005

Corresponding author: Frank M. Dugan — fdugan@wsu.edu Accepted for publication May 17, 2012.

Abstract: In 2003, 2008 and 2009 isolates of *Clonostachys* sp. were recovered from post-harvest chickpea debris. Representative isolates were identified as *C. rhizophaga* on the basis of 99% similarity of β-tubulin DNA sequences to those of the type strain and 100% similarity to representative strains, including isolates reported as highly aggressive on chickpea in Syria. In strong contrast to the report from Syria, our isolates of *C. rhizophaga* from the U.S. Pacific Northwest (PNW) did not induce severe wilt when artificially inoculated on seed of chickpea line ICC 12004, even at elevated temperatures. Two instances of wilt (1.3 % of all inoculated plants) and occasional, usually transient, negative effects on emergence were documented. Chickpea debris was dominated by common saprobic fungi in *Alternaria*, *Cladosporium* and *Ulocladium*. Mycoparasitic fungi isolated from chickpea debris included *C. rhizophaga*, *Cephalotrichum stemonitis* and *Harzia verrucosa*, the latter two documented from chickpea

for the first time. *C. rhizophaga* is already proven as mycoparasitic on *Ascochyta rabiei* (the primary fungal pathogen of chickpea), but *C. stemonitis* did not prove mycoparasitic on that fungus, and *H. verrucosa* did not remain viable in culture.

Key words: *Cephalotrichum stemonitis, Cicer arietinum, Clonostachys rhizophaga, C. rosea, Harzia verrucosa*, mycoflora, mycoparasitism, post-harvest debris.

Introduction: Current emphasis on reduced tillage practices presents challenges for chickpea producers in the Pacific Northwest (PNW) of the United States, because the region's most important pathogenic fungus on chickpea (*Cicer arietinum* L.), *Didymella rabiei* (Kovachevsky) Arx [conidial state, *Ascochyta rabiei* (Pass.) Lab.], overwinters in debris left on the soil surface. In the spring, *D. rabiei* produces ascospores that initiate Ascochyta blight epidemics (Milgroom and Peever 2003; Chilvers et al. 2007). Experimental inoculation of post-harvest debris with another fungal inhabitant of chickpea debris, *Aureobasidium pullulans* (de Bary) G. Arnauld, reduced subsequent Asochyta blight by 38% (Dugan et al. 2009). Another inhabitant of post-harvest debris, *Clonostachys* sp., was a mycoparasite of *D. rabiei* and could completely suppress both asexual and sexual reproduction of *D. rabiei in vitro* (Dugan et al. 2005).

Clonostachys spp., especially *C. rosea* (Link:Fr.) Schroers et al., have been used for experimental biological control, including control of diseases of chickpea (e.g., Burgess et al. 1997). Another species sometimes used for experimental biological control is a species related to *C. rosea*, *C. rhizophaga* (Tehon and Jacobs) Schroers et al. (Mendoza Garciá et al. 2003). This species was presented as the cause of a serious disease of elms in the original description, although Schroers (2001) expressed skepticism on this point. However, *C. rhizophaga* was later reported as causing severe wilt of chickpea in Syria (Abang et al. 2009). The species has subsequently been reported as causing disease of *Dendrocalamus* (a wild grass) in Mozambique (Zazzerinia and Quaglia 2010). We initially identified our isolates of *Clonostachys* as *C. rosea* (Link:Fr.) Schroers et al. on the basis of colony and morphological characters. Given the report of Abang et al. (2009), we resolved to reexamine our *Clonostachys* isolates with a molecular-genetic technique.

When post-harvest chickpea stem segments were plated to growth media, the most frequently encountered fungal taxa, in declining order, were various species of *Alternaria, Ulocladium, Epicoccum, Fusarium, Cladosporium, Phoma* and *Aureobasidium pullulans* (Dugan et al. 2005). The most common ascospores trapped from chickpea debris incubated in the laboratory were those of *Davidiella* spp. (teleomorphic *Cladosporium*) and *D. rabiei* (Dugan et al. 2009). We are highly aware that recovery of fungi is dependent on sampling protocol (Mueller et al. 2004), and regard our prior mycofloristic surveys (Dugan et al. 2005, 2009) as well as this one, as exploratory. Having previously performed such surveys by plating of tissues to agar media or trapping of ascospores (Dugan et al. 2005, 2009), we decided to use a third technique for this survey, incubation of post-harvest plant tissue in moist chambers, with the primary objectives of assessing abundance of *Clonostachys*, relative dominance of genera, and recovery of additional mycoparasitic fungi. We intended to further assess the mycoflora of chickpea debris, and to address potential pathogenic capabilities on chickpea for local populations of *C. rhizophaga* under conditions characteristic of the PNW, as well as under warmer conditions such as would approximate conditions in Syria, or in the PNW under climatic warming.

Materials and Methods:

Survey of post-harvest chickpea debris for mycoflora

Collection of materials: On 24 Oct 2008, we collected three sets of chickpea (Kabuli type) stems from experimental plots on the Spillman Agronomy Farm, near Pullman, WA: (A) on the ground since harvest of 22 September; (B) standing until 20 Oct when the field was tilled; and (C) standing until collected. On 28 Sept 2009, we collected four sets of stems from the same farm: (D and E) standing until collected; (F and G) on ground since harvest of 11 Sept; D and F were from the same area on the farm, E and G from another area.

Incubation, isolation, and examination of materials: After collection, all stems were stored at ambient lab temperatures for three days (2008) or for ten days, then at 5C for five days (2009), when they were trimmed into 3-5 cm segments. For each category (A-C, 2008; D-G, 2009) five segments were placed into each of ten 90 mm plastic culture dishes over blotter paper saturated with sterile distilled water. Dishes were wrapped in two layers of Parafilm® for retention of high humidity, and incubated for a minimum of 10 days under diurnal (12hr/12hr) near-ultraviolet/fluorescent lighting for promotion of sporulation. Fungi were then recovered to growth media by touching a sterile glass needle to areas of sporulation observed at 50X, streaking the spores onto Difco® malt extract agar for isolation of single colonies (each putatively from a single spore), and final transfer of single colonies to suitable growth media (Dugan et al. 2005) for storage and identification.

Identification of isolates: Representative *Clonostachys* isolates CB98B (from 2003, Dugan et al. 2005), and CP08C6 (from 2008), used in pathogenicity tests as described below, received preliminary identification as *Clonostachys* sp. on the basis of cultural and morphological characters (Schroers et al. 1999; Schroers 2001). We subjected these isolates, as well as CP63E, CP62B, and CP98E (from 2003), and CP09D2-5 (from 2009) to sequence analyses. DNA extraction and amplification of β-tubulin sequences followed Schroers (2001) and O'Donnell and Cigelnik (1997). Sequencing reactions and primer synthesis were performed by Eurofins MWG/Operon (Huntsville, AL). Sequence data were assembled and aligned using Sequencher™ 5.0 software (Gene Codes Corp., Ann Arbor, MI). The 600 nucleotide β-tubulin partial gene sequences amplified from our strains were aligned with corresponding sequences from Abang et al. (2009), and with type strains of *C. rhizophaga* and *C. rosea* (Shroers 2001) using BLASTN 2.2.21 software (Zhang 2000). Taxa already well documented as dominant in chickpea debris in prior surveys (*Alternaria*, *Cladosporium*, *Epicoccum, Fusarium* and *Ulocladium*) were neither isolated nor identified to species, but their presence was diagnosed at 50X, confirmed as needed at 100-400X and recorded for each occurrence in the culture dishes of sets A-C, D-G. Literature used for morphological identification of isolates to genus and/or species consisted of Barron (1968), Domsch et al. (1993), and Schroers (2001), with nomenclature updated as necessary (www.indexfungorum.org).

Pathogenicity tests: *Clonostachys* isolates CB98B and CP08C6 were tested by procedures analogous to Burgess et al. (1997), who used suspensions of an isolate they identified as *C. rosea* to inoculate chickpea seeds as protection against infection by *Botrytis cinerea* Pers.

Seeds of chickpea line ICC 12004 (a line which suffered 100% mortality from wilt induced by *C. rhizophaga* in Abang et al. 2009) were surface-disinfested in 0.5% sodium hypochlorite, rinsed in sterile distilled water, soaked for 2 hours in a suspension of 10^6 conidia/ml of either CB98B or CP08C6, dried overnight, and planted in pre-moistened Sunshine™ potting soil. We used 5 seeds per pot and 5 pots per treatment (5 pots each for CB98B and CP08C6, and two controls of

water soak only, each also with 5 pots containing 5 seeds each). Beginning 24 May 2009, plants were reared in the greenhouse under natural daylight + supplemental lighting of 400 watt high pressure sodium lamps to extend daylight to 18 hrs and temperatures of ca. 22-30C days and 16C nights; pots were placed in a randomized design. Plants were grown for 16 days, when results were recorded. The experiment was repeated in 2010, beginning 20 Jan; plants were permitted to grow until flowering.

In 2011, an analogous trial using the two isolates (5 pots for each isolate, each pot with 5 seeds as previously described, with 5 pots of corresponding mock-inoculated controls) was conducted in a growth chamber, with daytime temperatures of 22-34C days (34C for minimum of 4 hr) and 18C nights, to more closely approximate Syrian field conditions, or those of eastern Washington under modest climatic warming, and under artificial lighting with a combination of high pressure sodium and metal halide HID lights to simulate 14 hr days. The experiment was run until pod set. Data for all experiments were analyzed with Systat 10.2 (GLM, LSD) with each pot treated as a replication and individual pair-wise comparisons conducted as appropriate.

Results: *Survey of post-harvest debris*: No differences were apparent between sets of stems A-C in 2008, nor between sets of stems D-G in 2009, with respect to predominant genera. *Alternaria*, *Cladosporium*, and *Ulocladium* (in order of decreasing abundance) were the most conspicuous genera in both years and in all sets of stems. However, in 2008 *Clonostachys* was observed in 10 of 30 incubation dishes (3 of 10 in set A, 5 of 10 in set B, 2 of 10 in set C), whereas in 2009 it was observed in only 1 of 40 dishes (1 of 10 in set G). Additional conspicuous genera in 2008 and 2009 (most isolates conserved but not identified to species) were *Acremonium* (series Plectonematogen, sensu Gams 1971), *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Stemphylium*. *Penicillium* and *Rhizopus* were especially common in 2009. Not surprisingly, *A. rabiei* formed a component of debris mycoflora in both 2008 and 2009. *Papulospora* sp. was isolated in 2008 and *Aspergillus* in the *A. nidulans* group was especially notable in 2008. Additional taxa isolated in 2009 included the potential mycoparasites *Cephalotrichum stemonitis* (Pers.) Nees [= *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm.] and *Harzia verrucosa* (Tognini) Hol.-Jech. (= *Acremoniella verrucosa* Tognini).

Isolates CP08C6 (from 2008) and CB98B (from Dugan et al. 2005) were identified as *C. rhizophaga* on the basis of β-tubulin sequences, although these isolates had produced abundant secondary conidiophores (Fig. 1), spore mass coloration of light orange, and conidia of high length to width (L/W) ratios (up to 2.7), all congruent with descriptions of *C. rosea* (Schroers et al. 1999; Schroers 2001). β-tubulin DNA sequences of both isolates displayed 99% homology with type material (CBS 202.37) of *C. rhizophaga* versus 94% homology with type material (CBS 710.86) of *C. rosea*.

C. rhizophaga isolates CP08C6, CP98B, as well as CP63E, CP62B, and CP98E (from 2003), and CP09D2-5 (from 2009) were 100% identical to each other in the β-tubulin 600 nucleotide partial gene sequence. Our sequences were 100% identical to the *C. rhizophaga* strains found to be pathogenic to chick pea in Abang et al. (CBS 124507 and CBS 124511) and were 99% identical to type material for *C. rhizophaga* (CBS 202.37), with our strains having a single two- base deletion compared to the type sequence. Our strains are also 100% identical to *C. rhizophaga* strains CBS 361.77 and CBS 100004 (Schroers 2001) and 99% identical with a single-base change at position 231 in CBS 906.72a (Schroers 2001). Sequences of CP08C6, CB98B and CP63E are deposited with GenBank as numbers JX025637, JX025636, and JX025635, respectively.

Pathogenicity tests: In 2009, neither *Clonostachys* isolate induced wilt, but both initially inhibited emergence (Fig. 2). Initially, overall means were 3.0 emerged plants/pot in treatments versus 4.7 in controls ($P < 0.04$, 11 days post-planting). Within individual pair-wise comparisons, isolate CP08C6 differed from control #1 (mean = 4.4) only at a very marginal $P = 0.13$; CP98B differed from both controls at $P \leq 0.05$. With emergence of more plants in treatments at 16 days, CP98B (mean = 3.0) but not CP08C6 (mean = 3.4) differed from both controls (means = 4.4, 5.0), but only marginally at $P \leq 0.09$. In 2010, emergence was again initially inhibited in treatments versus controls for both isolates, but by the end of the experiment only for CP08C6: At 8 days post-planting, the numbers of emerged plants/pot for CP98B (mean = 2.6) and CP08C6 (mean = 3.0) were less than for controls (means of 4.8, 4.6) ($P \leq 0.01$). However, at 16 days CP08C6 (mean = 3.8) differed ($P < 0.03$) from both controls (means = 4.8, 5.0), but not so with CP98B (mean = 4.8). Notably, one plant inoculated with CP98B and one inoculated with CP08C6 subsequently wilted just after emergence, with *C. rhizophaga* being re-isolated in both instances. In 2011, neither isolate affected emergence, nor induced wilt, nor affected appearance of roots by the time trials were terminated after pod formation (Fig. 3).

Discussion: *Clonostachys rhizophaga* was repeatedly isolated from chickpea debris in this study as well as in Dugan et al. (2005), and is documented as inducing severe wilt (Abang et al. 2009) or sporadically reducing and retarding emergence in chickpea (this study). Although Abang et al. (2009) implicated *C. rhizophaga* as causing disease in the field in Syria, there are to date no analogous reports documenting disease in the field caused by *Clonostachys* on chickpea elsewhere. Schroers (2001) and Hepting (1971) reviewed, somewhat skeptically, the report of *C. rhizophaga* causing disease of *Ulmus americana* (elm) in the original (and invalid, no Latin diagnosis) description of the fungus.

Clonostachys species, including *C. rhizophaga*, are mycoparasites and have been repeatedly used as experimental biological control agents. Recent examples include Cota et al. (2008) using *C. rosea* for control of *Botrytis* on strawberry, and Jensen et al. (2004) using *C. rosea* for control of *Alternaria* on carrot. *C. rosea* has also been used for experimental control of fungal disease of legumes. Burgess et al. (1997) used *C. rosea* to control *Botrytis* on chickpea, and Xue (2003) for control of various root rot pathogens of field pea. Our isolates of *C. rhizophaga* (previously determined as *C. rosea* on the basis of morphological and cultural characters) have been demonstrated as mycoparasitic on *Didymella rabiei in vitro* (Dugan et al. 2005). *C. rhizophaga* has also been used to control Rosellinia root rot of cocoa (Mendoza Garciá 2003).

Given the devastating impact of *C. rhizophaga* on chickpea in Syria and given that *Clonostachys* spp. are common soil fungi occasionally indicated as seed-borne (e.g., IMI 341053, 363644 in Kew Botanic Gardens n.d.; Schroers 2001) consistent isolation of *C. rhizophaga* from post-harvest debris had implications for the health and regulatory status of chickpea from Washington State. Given the sporadic reduction or retardation in emergence of chickpea in artificial inoculations (this study), and given the record of *Clonostachys* species (including *C. rhizophaga*) as mycoparasites, the overall impact of *C. rhizophaga* on chickpea production in the PNW remains ambiguous. However, the failure of isolates of *C. rhizophaga* from the PNW to consistently induce wilt, reduce emergence, or affect roots bodes well for chickpea production in the PNW. The contrast with the situation in Syria, however, raises questions about variation within *C. rhizophaga* and/or its response to environmental conditions. It is plausible that the impact is negative under some conditions as demonstrated experimentally here (albeit

inconsistently, and with minor effects) or, much more consistently and with major impact, in Abang et al. (2009) under Syrian conditions. On the other hand, mycoparasitism of *C. rhizophaga* on fungal pathogens such as *A. rabiei* might, under other conditions, render the impact positive, as demonstrated *in vitro* (Dugan et al. 2005).

The isolates in our study exhibited a high frequency of secondary conidiophores, light orange conidial masses, and high conidial L/W ratios (Dugan et al. 2010). Okuda et al. (2000) noted variation in characters, including conidial shape, amongst various "groups" of isolates assigned by them to *C. rosea*, with one isolate (TC 1282, their Fig. 4F) displaying L/W ratios up to 2.8. These attributes are more in conformity with the most comprehensive published description of *C. rosea* than with the corresponding description for *C. rhizophaga* (Schroers et al. 1999; Schroers 2001). However, Schroers (2001) refrained from separating these species in his morphological key because of "the rather cryptic characters" and based his separation "mainly on DNA sequence analysis." The validity of his decision not to separate these species on the basis of morphology is confirmed in this study.

Clonostachys rhizophaga (*Verticillium rhizophagum* Tehon & Jacobs, described in 1936 in a relatively obscure publication, Bulletin of The Davey Tree Expert Company) was not a widely known fungus until reassigned to *Clonostachys* by Schroers (2001). It is now apparent that the fungus is widely distributed and more common than reports previous to 2001 would indicate. At least one study (Piontelli and Guisiano 2003), reported both *C. rosea* and *C. rhizophaga* from a single substratum (needles of *Pinus canariensis* C. Smith). Apparently identified on cultural and morphological criteria, the isolates assigned to *C. rosea* by Piontelli and Guisiano (2003) produced orange conidial masses, whereas those assigned to *C. rhizophaga* lacked this coloration. Two isolates previously considered as *C. rosea* on the basis of orange coloration, frequency of secondary conidiophores, and high L/W ratio of conidia and used for biological control *in vitro* (Dugan et al. 2005), were actually *C. rhizophaga*, assuming β-tubulin sequence is the preferred diagnostic criterion. Other pertinent instances of putative *C. rosea* being more accurately assigned to *C. rhizophaga* include CBS 229.48, isolated from soil in Washington State in 1930 and originally identified as *Gliocladium roseum* Bain (= *C. rosea*), and CBS 529.80 isolated from *Pisum sativum* L. in Ecuador (www.cbs.knaw.nl/collections, 22 Feb 2012). Both were re-identified as *C. rhizophaga* by Schroers (2001) along with other strains originally assigned to *C. rosea*. Results here, in the context of Dugan et al. (2005, 2010), broaden the morphological/cultural limits for *C. rhizophaga* with respect to coloration of conidial masses, conidial L/W ratios and frequency of secondary conidiophores.

There may also be variation in taxonomic opinion. A major culture collection (Belgian Co-ordinated Collections of Microorganisms, BCCM, http://bccm.belspo.be, 22 Feb 2012) documents the name *C. rhizophaga*, but catalogs the type strain of *Verticillium rhizophagum* under the names *Nectria ochroleca* (Schwein.) Berkeley (a teleomorphic name) and *Gliocladium roseum* (an anamorphic name). This catalog also provides for other strains of *Nectria ochroleuca* the name *Verticillium rhizophagum* as a synonym. The name *G. roseum* is a synonym of *C. rosea*, and *Nectria ochroleuca* is a synonym of *Bionectria ochroleuca* (Schwein.) Schroers & Samuels, the teleomorph of *C. rosea* (Schroers et al. 1999). BCCM is a major collection with trained fungal taxonomists and the synonymy implies a taxonomic opinion at BCCM in favor of a broader species concept. But, to our knowledge there is no opinion published under peer review regarding *C. rhizophaga* and *C. rosea* as synonyms, and the phylogenetic trees in Schroers (2001) can be interpreted as evidence against synonymy. It

should be noted that our strains of *Clonostachys* exhibited 99% sequence homology with the type strain of *C. rhizophaga*, and strains in Abang et al. (2009) exhibited 100% homology to an authentic strain but differed by 2 nucleotides from the type strain. Establishing the full range of morphological and molecular-genetic variation within these closely related fungi, and establishing agreement on species concepts applicable to *Bionectria*/*Clonostachys*, may either confirm or revise taxonomic opinion. In any case, persons conducting research on chickpea or other legumes should document further observations on interactions of *Clonostachys* species with legume crops.

Taxa documented in this study on stems (*Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, and *Ulocladium*) were also highly apparent in results of Dugan et al. (2005), who plated stem segments to growth media, but *Cladosporium* was more frequent than in Dugan et al. (2005). Other fungi of interest included *Cephalotrichum stemonitis*, isolated by us in 2009 and previously reported as associated with stalk rot of maize (Gulya et al. 1979). This species has also been associated with 'speck rot' or 'brown rot' of potato, but has been demonstrated to be a mycoparasite of *Pythium ultimum* Trow. and *Fusarium coeruleum* (Lib.) Sacc. in rotting potatoes, and may actually "be occurring as a hyperparasite on some fungus inciting decay" (Thirumalachar and Pavgi 1963). It is also recorded as a saprobe on a wide variety of plants and appears to be globally distributed (Farr and Rossman n.d.). We did not detect mycoparasitism of *A. rabiei* by *C. stemonitis* when paired isolates were grown on agar-covered slide cultures (data not shown). *Harzia verrucosa*, isolated by us in 2009, also possesses mycoparasitic abilities, illustrated by parasitism on *Dendryphiella salina* (G.K. Sutherl.) Pugh & Nicot (Malloch n.d.). In addition to its presence on plants listed by Farr and Rossman (n.d.), *H. verrucosa* has been reported in rice field soil in California (Keim et al. 1975), in estuarine sediment in North Carolina (Borut and Johnson 1962), in flax seed in Lithuania (Gruzdevienė et al. 2006), from grapes in France (Sage et al. 2002), coniferous litter in Argentina (Piontelli et al. 2005), in seeds of desert shrubs in Namibia (Piontelli et al. 2004), from dung of various herbivores in Kenya (Caretta et al. 1998), and as a member of the aerobiota in Egypt (Ismail et al. 2002). This distinctive fungus appears to be plurivorous and of global distribution; see also Barron (1968) and Ellis (1971) for additional substrata. We did not observe the microconidial state illustrated by Matsushima (1975), and our isolate became nonviable after serial transfer. To our knowledge, this is the first report of *C. stemonitis* and *H. verrucosa* from chickpea.

Acknowledgements: The authors would like to acknowledge Donna Ayling and Ellen Johnston for technical assistance, and Lyndon Porter and Kurt Schroeder for helpful comments on the manuscript.

Literature cited

Abang, M.M., S. Kabbabeh, S. Ahmed, S. Murad, M.I. Chilvers, T.L. Peever, and H.-J. Schroers. 2009. First report of chickpea wilt caused by *Clonostachys rhizophaga* in Syria. Plant Disease 93: 666. dx.doi.org/10.1094/PDIS-93-6-0666A

Barron, G.L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins, Baltimore. 364 pp.

Borut, S.Y., and T.W. Johnson, Jr. 1962. Some biological observations on fungi in estuarine sediments. Mycologia 54: 181-193. dx.doi.org/10.2307/3756668

Burgess, D.R., T. Bretag, and P.J. Keane. 1997. Biocontrol of seedborne *Botrytis cinerea* in chickpea with *Gliocladium roseum*. Plant Pathology 46: 298-305. dx.doi.org/10.1046/j.1365-3059.1997.d01-17.x

Caretta, G., E. Piontelli, E. Savino, and A. Bulgheroni. 1998. Some coprophilous fungi from Kenya. Mycopathologia 142: 124-134. dx.doi.org/10.1023/A:1006995313657

Chilvers, M., T. Peever, H. Akumatsu, W. Chen, and F.J. Muehlbauer. 2007. *Didymella rabiei* primary inoculum release from chickpea debris in relation to weather variables in the Pacific Northwest of the United States. Canadian Journal of Plant Pathology 29: 365-371. dx.doi.org/10.1080/07060660709507482

Cota, L.V., L.A. Maffia, E.S.G. Mizubuti, P.E.F. Macedo, and R.F. Antunes. 2008. Biological control of strawberry gray mold by *Clonostachys rosea* under field conditions. Biological Control 46: 515-522. dx.doi.org/10.1016/j.biocontrol.2008.04.023

Domsch, K.H., W. Gams, and T.-H. Anderson. 1993. Compendium of Soil Fungi (vols. 1, 2). IHW-Verlag, Eching. 860 pp, 406 pp.

Dugan, F.M., H. Akamatsu, S.L. Lupien, W. Chen, M.L. Chilvers, and T.L. Peever. 2009. Ascochyta blight of chickpea reduced 38% by application of *Aureobasidium pullulans* (anamorphic Dothioraceae, Dothideales) to post-harvest debris. Biocontrol Science & Technology 19: 537-545. dx.doi.org/10.1080/09583150902730448

Dugan, F.M., S.L. Lupien, and W. Chen. 2010. *Clonostachys* in chickpea debris in the Palouse region of the Pacific Northwest, USA. Proceedings of the 2nd International Ascochyta Workshop, 28 June - 2 July 2009, Pullman, WA. Plant Management Network, www.plantmanagementnetwork.org/proceedings/Ascochyta/2009.

Dugan, F.M., S.L. Lupien, M. Hernandez-Bello, T.L. Peever, and W. Chen. 2005. Fungi resident in chickpea debris and their suppression of growth and reproduction of *Didymella rabiei* under laboratory conditions. Journal of Phytopathology 153: 431-439. dx.doi.org/10.1111/j.1439-0434.2005.00996.x

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Agricultural Institute, Kew, Surrey, England. 608 pp.

Farr, D.F., and A.Y. Rossman. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved December 9, 2009, from http://nt.ars-grin.gov/fungaldatabases.

Gams, W. 1971. Cephalosporium-artige Schimmelpilz (Hyphomycetes). Gustav Fischer, Stuttgart. 262 pp.

Gruzdevienė, E., A. Mankevičienė, A. Lugauskas, and J. Repečkienė. 2006. The effect of environmental conditions on the variation of fungi and mycotoxin contents in oil flax seed. Ekologija 3: 64-70.

Gulya Jr., T.J., C.A. Martinson, and L.H. Tiffany. 1979. Ear-rotting fungi associated with opaque-2 maize. Plant Disease Reporter 63: 370-373.

Hepting, G.H. 1971. Diseases of Forest and Shade Trees of the United States. USDA Forest Service, Agricultural Handbook 386.

Ismail, M.A., S.I.I. Abdel-Hafez, and A.M. Moharram. 2002. Aeromycobiota of western desert of Egypt. African Journal of Science and Technology, Science and Engineering Series 3: 1-9.

Jensen, B., I.M.B. Knudsen, M. Madsen, and D.F. Jensen. 2004. Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seedborne *Alternaria* spp. Phytopathology 94: 551-560. dx.doi.org/10.1094/PHYTO.2004.94.6.551

Keim, R., R.K. Webster, and C.M. Wick. 1975. Quantitative effects of incorporating rice residue

on populations of soil microflora. Mycologia 67: 280-292. dx.doi.org/10.2307/3758420

Kew Botanic Gardens. n.d. Herb IMI records. www.herbimi.info/herbimi/results.htm?l2code=40&page=102; www.herbimi.info/herbimi/results.htm?iso=CM, retrieved 25 May 2011.

Malloch, D. n.d. Fungi parasitic on other fungi. New Brunswick Museum, Saint John, New Brunswick, Canada. http://website.nbm-mnb.ca/mycologywebpages/NaturalHistoryOfFungi/FungalParasites.html

Matsushima, T. 1975. Icones Microfungorum a Matsushima Lectorum. Published by the author, Kobe, Japan. 209 pp + 415 plates.

Mendoza Garciá, R.A., G. Martijn ten Hoopen, D.C. J. Kass, V.A. Sánchez Garita, and U. Krauss. 2003. Evaluation of mycoparasites as biocontrol agents of *Rosellinia* root rot in cocoa. Biological Control 27: 210-227. dx.doi.org/10.1016/S1049-9644(03)00014-8

Milgroom, M.G., and T.L. Peever. 2003. Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. Plant Disease 87: 608-617. dx.doi.org/10.1094/PDIS.2003.87.6.608

Mueller, G.M., G.F. Bills, and M.S. Foster. 2004. Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier, Amsterdam. 777 pp.

O'Donnell, K., and E. Cigelnik. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116.

Okuda, T., J. Kohno, N. Kishi, Y. Asai, M. Nishio, and S. Komatsubara. 2000. Production of TMC-151, TMC-154 and TMC-171, a new class of antibiotics, is specific to '*Gliocladium roseum*' group. Mycoscience 41: 239-253. dx.doi.org/10.1007/BF02489678

Piontelli, E.L., and G. Giusiano. 2003. Notas micologicas VI: seleccion de microhongos asociados a material vegetal y queratina desde Argentina y Chile. Boletin Micológico 18: 89-99.

Piontelli, E., G. Giusiano, and D. Casanova. 2005. Comunidad estacional de microhongos en la litera de coniferas del N.E. Argentino: enfasis en taxa de potencial interes medico. Boletin Micológico 20: 15-27.

Piontelli, E., G. Giusiano, E. Savino, N. Solari, and G. Caretta. 2004. Microfungi from different substrata in South West Africa (Namibia). Boletin Micológico 19: 63-69.

Sage, L., S. Krivobok, É. Delbos, F. Seigle-Murandi, and E. Creppy. 2002. Fungal flora and ochratoxin A production in grapes and musts from France. Journal of Agricultural and Food Chemistry 50: 1306-1311. dx.doi.org/10.1021/jf011015z

Schroers, H.-J. 2001. A monograph of *Bionectria* (Ascomycota, Hypocreales, Bionectriaceae) and its *Clonostachys* anamorphs. Studies in Mycology 46: 1-214.

Schroers, H.-J., G.J. Samuels, K.A. Seifert, and W. Gams. 1999. Classification of the mycoparasite *Gliocladium roseum* in *Clonostachys* as *C. rosea*, its relationship to *Bionectria ochroleuca*, and notes on other *Gliocladium*-like fungi. Mycologia 90: 365-385. dx.doi.org/10.2307/3761383

Thirumalachar, M.J., and M.S. Pavgi. 1963. *Stysanus stemonites* (Pers.) Corda on potato in cold store in India. Cellular and Molecular Life Sciences [Experientia] 19(2): 86.

Xue, A. G. 2003. Biological control of pathogens causing root rot complex in field pea using

Clonostachys rosea strain ACM941. Phytopathology 93:329-335. dx.doi.org/10.1094/PHYTO.2003.93.3.329

Zazzerini, A., and M. Quaglia. 2010. First report of *Clonostachys rhizophaga* as a pathogen of *Dendrocalamus giganteus* in Mozambique. Plant Disease 94: 372. dx.doi.org/10.1094/PDIS-94-3-0372C

Zhang, A., S. Schwartz, L. Wagner, and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7: 203-214. dx.doi.org/10.1089/10665270050081478

Figs. 1-3. *Clonostachys rhizophaga* from the U.S. Pacific Northwest (PNW) and its inconsistent effects on emergence of chickpea. Fig.1. Secondary conidiophores and conidia of *Clonostachys rhizophaga* CP98B (from oatmeal agar). Differential interference contrast. Bar = 10 µm. Fig. 2. Plants from seed inoculated with isolate CP08C6 (right) occasionally showed reduced emergence relative to controls (left) under conditions approximate to those in the PNW. Fig. 3. Plants artificially inoculated and incubated under conditions approximate to those in Syria; treatments and controls (shown randomized in chamber) were equally asymptomatic and vigorous.