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Diseases and Disease Management in Seed Garlic: Problems and Prospects

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ABSTRACT

Although garlic is occasionally propagated via true seed, routine planting of garlic uses seed cloves as vegetative propagules. The size of seed cloves (large relative to seed of most agronomic crops), their vegetative habit, and routine storage conditions for seed cloves (permissive for most fungi), create opportunities for pathogens and problems for growers. Several phytopathogenic fungi, including some newly documented as pathogenic to garlic, are able to infest or colonize bulb tissues and remain latent for some time subsequent to harvest. Infested or infected bulbs may appear healthy at time of shipping or receipt, and even for protracted periods of storage, but incubation at suitable temperatures can result in the appearance of rot. The potential for planting seed cloves containing pathogens, plus the capacity of several of these fungal pathogens for prolonged survival in field soil, implies that pathogens may be introduced into and contaminate field soils. Systemic fungicides used as pre-planting and/or post-harvest dips can promote plant health, but the large size of seed cloves insures that deep-seated infections are not eradicated. Viruses also persist in vegetative material, are unaffected by fungicides, have been detected in a high proportion of garlic grown as planting stock, and often have arthropod vectors that are difficult to control. To circumvent these problems, tissue culture is increasingly used to generate disease free planting stock.

Keywords: *Allium sativum*, fungi, garlic, pathogens, seed clove, viruses

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INTRODUCTION

Some varieties of garlic (*Allium sativum* L.) can be propagated by true seed (Simon and Jenderek 2003), a trait extremely useful for the breeder. Production of true seed from garlic is the subject of U.S. Patent 5746024. However, the most common method for growing garlic involves planting of clonal propagules, i.e., seed cloves (Sims *et al*. 1976; Sutherland and Waverley 1995; Hannan and Sorensen 2002), or in some instances, inflorescence bulbils (Schwartz *et al*. 1995; Simon and Jenderek 2003). This review primarily addresses management of diseases affecting or transmitted by seed cloves. Typically, these cloves originate with plants harvested in late summer (e.g., early July to mid-September in the Pacific Northwest of the USA), then stored and planted again in fall (mid-September to November) or for certain varieties early spring (March) (Hannan and Sorensen 2002; Barbara Hellier, pers. comm.). Storage conditions of approximately 12-14ºC and 55% relative humidity retard sprouting during storage and optimize rapid germination and growth upon planting (Hannan and Sorensen 2002). Others recommend a storage temperature closer to 10ºC, but holding bulbs near 5ºC for extended times increases risk of sprouting (Sutherland and Waverley 1995). Cloves destined for spring-planting have been stored at 0ºC or -3ºC with successful results, but some cultivars are sensitive to such low temperatures (Volk and Rotindo 2004).

THE SEED CLOVE AS HABITAT FOR PESTS AND PATHOGENS

The large size of the clove (relative to the true seed of most other field crops) and storage at ca. 10-14ºC create difficulties in disease management. Cloves are sufficiently large that even prolonged exposure to systemic fungicides in pre-planting or post-harvest dips is insufficient to eliminate deeply seated infections (Dugan *et al*. in press), so unlike most true seed the use of fungicidal dips is no guarantee of

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strongly reduced inoculum in planting stock. Fungicidal dips are effective only on recently established, relatively shallow infection courts, although systemic fungicides are labeled for use against *Fusarium* and *Penicillium* bulb rots in ornamental flowers as well as in *Allium* species (G. Chastagner pers. comm.; Hong 2007).

Attacks by bulb mites (*Rhizoglyphus* spp. and *Tyrophagus* spp.) and the wheat curl mite (*Eriophyes tulipae* Keifer) in storage can also promote rot. Just as the above storage conditions are permissive for many fungi, they are also permissive for mites (Coviello *et al*. 2002). *Rhizoglyphus robini* Claparede, *Tyrophagus putrescentiae* Shrank and/or *Histiostoma onioni* Eraky were demonstrated capable of vectoring *Aspergillus niger* Van Tieghhem, *A. ochraceus* Wilhelm, *Fusarium oxysporum* Schlechtend. : Fr., *Gibberella fujikuroi* (Sawada) Ito, *Penicillium* spp. and other fungi (Abdel-Sater and Eraky 2001). Mite control is difficult, even with chemicals. A comprehensive guide to diseases and pests of garlic is available (Schwartz and Mohan, in press).

FUNGAL PATHOGENS OF SEED GARLIC

The most common fungal pathogens attacking garlic bulbs in storage are *Aspergillus niger*, *A. ochraceus*, *Embellisia allii* (Campanile) Simmons, *Fusarium oxysporum* Schlechtend. : Fr. f. sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans. *F. proliferatum* (Matsushima) Nirenberg (**Fig. 1**), and *Penicillium hirsutum* Dierckx (**Fig. 2**). We have also found instances of rot involving *Botrytis porri* Buchw., and the recently reported *Fusarium verticillioides* (Sacc.) Nirenberg (Dugan *et al*., in press). However, not all isolates to which these names can be applied are necessarily aggressive. We have isolates of *A. ochraceus*, *A. niger* and *E. allii* that are not very aggressive in comparison to other pathogenic species (although *E. allii* especially has been quite damaging under moist field conditions). Moreover, we have evidence that some isolates of *Fusarium oxysporum* f. sp. *cepae* and *F. proliferatum*, quite aggressive in onion, are less aggressive in garlic, especially when the latter has aged ("hardened") subsequent to harvest (Dugan *et al*. in press; Slavica *et al*. in press). The extent to which some of these pathogens might remain quiescent in tissues for one or more clonal generations, similar to the situation documented by Crowe (1995) for *Fusarium culmorum* (Wm. G. Sm.) Sacc., is largely unknown. However, in a survey of asymptomatic, commercially distributed seed garlic, Dugan *et al*. (in press) recovered three or more of these pathogenic species from each of seven lots: six lots from various states in the USA and one lot from mainland China. Molecular-genetic protocols for detection and differentiation of some mycotoxin-producing fungi documented from garlic have been published (Mulè *et al*. 2004).

One of the most aggressive agents of rot in garlic is a species of *Penicillium*, the correct name for which is the subject of recent publications. The name *P. corymbiferum* (= *P. verrucosum* var. *corymbiferum* (Westling) Samson, Stolk & Hadlok) was formerly often applied (e.g., Smalley and Hansen 1962; Brammall 1989) but has been largely replaced by its synonym *P. hirsutum* (Pitt 2000). In fact, various names have been applied to *Penicillium* species rotting garlic (Brammall 1989). Overy *et al*. (2005) examined a collection of several isolates, amongst which only those bearing the name *Penicillium allii* Vincent & Pitt were strongly pathogenic to garlic; isolates bearing the name *P. hirsutum* were not very aggressive. Valdez *et al*. (2006) applied the name *P. allii* to isolates pathogenic to garlic in Argentina. Cavagnaro *et al*. (2005) use the name *P. hirsutum*. Dugan *et al*. (in press) noted such varied preferences, but applied the name *P. hirsutum* in a broad sense to all the isolates pathogenic to garlic in their work because some produced a deeply colored exudate said to characterize that species (Frisvad and Samson 2004). Several species in section Corymbifera, including *P. allii*, were formerly treated as varieties of *P. hirsutum*, including *P. hirsutum* var. *allii*

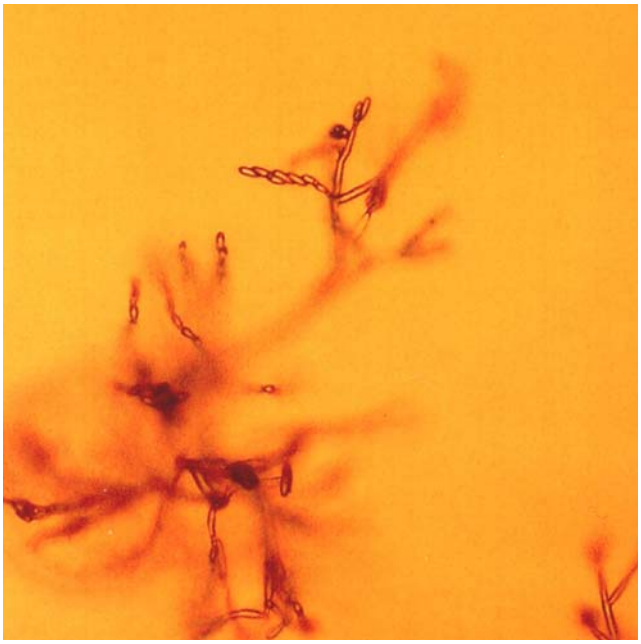

Fig. 1 Imbricate chain of conidia of *Fusarium proliferatum* on potassium chloride agar.

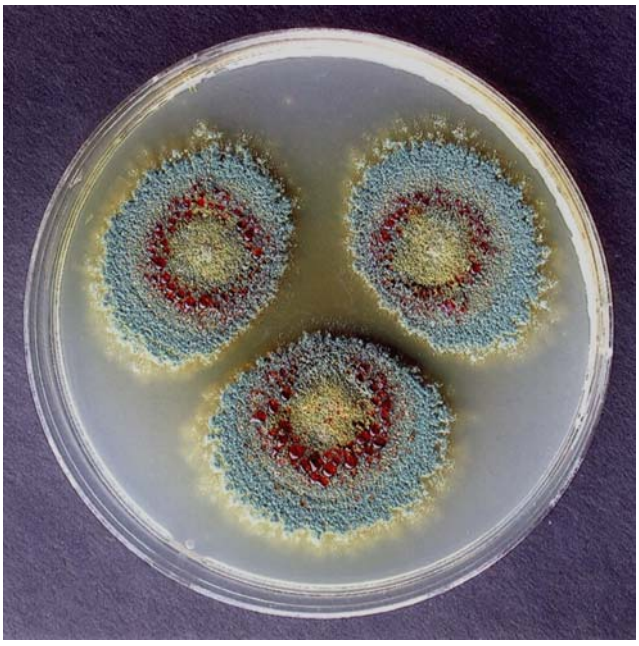

Fig. 2 An isolate of *Penicillium hirsutum* with deep red exudate on Czapek's agar.

(Vincent & Pitt) Fisvad (Frisvad and Filtenborg 1989). Living type material (strictly speaking, ex-type) of *P. allii* is from garlic, but type material for *P. hirsutum* is a neotype isolated from aphids (Frisvad and Samson 2004).

Sclerotium cepivorum Berk., the white rot fungus, is a severe problem in field garlic, and is the object of quarantine for seed cloves in some locales (Hannan and Sorensen 2002). Hot water treatments (see below) can be effective in managing, but probably not eradicating, *S. cepivorum* in planting stock (Sims *et al*. 1976; Anon 2004; Davis *et al*. 2005).

SURVIVAL OF FUNGAL PATHOGENS IN SOIL

Fusarium oxysporum. f. sp. *cepae* produces chlamydospores, and is capable of protracted survival (Havey 1995). *F. proliferatum* does not produce chlamydospores (Nelson *et al*. 1983) but was capable of prolonged survival in soil when associated with residue (Cotton and Munkvold 1998). Dugan *et al*. (in press) found that *F. proliferatum* and *F.*

oxysporum in field soil survived prolonged freezing in simulated winter conditions. *Fusarium verticillioides* is also documented as surviving well in field conditions (Leslie and Summerell 2006). *E. allii* is reported to over-winter in plant debris or soil (David 1991). *B. porri* produces sclerotia of considerable size (Chilvers and du Toit 2006), and *A. ochraceus* can also produce sclerotia (Klich 2002). As its name indicates, *Sclerotium cepivorum* also produces sclerotia, and a number of management strategies have evolved to facilitate their reduction, e.g. compounds that stimulate germination by mimicking exudates of *Allium* roots (Hovius and McDonald 2002; Davis *et al*. 2007). Sclerotia of *S. cepivorum* can persist for years, even in absence of the host (Maria Jenderek, pers. comm.). *Penicillium hirsutum*, however, does not persist for a long time in soil (Anon 2004).

MYCOTOXIN PRODUCTION

Although this review primarily addresses seed garlic, it is important to note that several fungi produce toxins which might become important in table garlic. Seefelder *et al*. (2002) claimed detection of fumonisin mycotoxins by *F. proliferatum* in market garlic in Germany, and the pathogen is now reported from garlic in North America (Dugan *et al*. 2003; du Toit and Dugan, in press). *F. verticillioides* has only recently been documented as rotting garlic (Dugan *et al*., in press), but much has been written on mycotoxin production by *F. verticillioides* (e.g., Shim and Woloshuk 2001). "*F. proliferatum* and *F. verticillioides* are the two most prolific producers of fumonisins" and *F. proliferatum* produces additional mycotoxins (Desjardins 2006). Mycotoxin production by *Allium*-inhabiting isolates of *F. proliferatum* has been analyzed (Stankovic 2007) Some isolates of *Aspergillus ochraceous* and *A. niger* produce ochratoxin A (Klich 2002). *Penicillium hirsutum* may produce roquefortine C, and *P. allii* is also documented as producing this compound (Frisvad and Samson 2004).

BACTERIA

Pseudomonas fluorescens Migula has been documented as pathogenic in garlic and causing a disease named 'maladie café au lait' in France (Diekmann 1997) and *Burkholderia cepacia* (Palleroni and Holmes) Yabuuchi *et al*. is a regulated organism on at least one pest list for garlic (Herrera 2005). In addition, *Erwinia carotovora* ssp. *carotovora* (Jones) Bergey *et al*., *E. chrysanthemi* Burkholder *et al*., *Pseudomonas gladioli* Severini and *Enterobacter cloacae* (Jordan) Hormaeche and Edwards are specified as causing soft rot of onion and garlic; however, these "are primarily a problem on onions, but not garlic" (Davis *et al*. 2005).

NEMATODES

Ditylenchus dipsaci (Kühn) Filipjev is a major pest of *Allium* spp. throughout temperate climes (Diekmann 1997; Hannan and Sorensen 2002; Anon 2004). Hot water treatments, especially if preceded by prolonged soak in cold water, are effective (Sims *et al*. 1976; Johnson and Roberts 1995; Diekmann 1997), although prior experimentation is prudent because garlic cultivars differ in ability to withstand higher temperatures without injury (Anon. n.d.). It is necessary to find temperatures sufficiently high to kill the nematode but not so high as to cause serious damage to the garlic host. In the absence of considerable amplification, many accessions of germplasm have insufficient material for the necessary experimentation, but hot water treatments can be useful for situations in which there is abundant production of a given variety.

VIRUSES

Virus infection is a problem for garlic germplasm production, not only because of reductions in yield or quality, but because mild virus symptoms may be confounded with varietal differences within garlic germplasm. Viruses especially notable as infecting garlic include: *Onion yellow dwarf virus* (OYDV, transmitted in a non-persistent manner by *Myzus persicae* and several other aphid species), *Leek yellow stripe virus* (LYSV, aphid transmitted in a non-persistent manner by several species of aphids), *Garlic common latent virus* (GCLV, transmitted by mechanical inoculation and aphids) and *Shallot latent virus* (SLV, transmitted in a non-persistent manner by *Myzus* (*Sciamyzus*) *ascolonicus* and, perhaps, *Aphis fabae*) (Brunt *et al*. 1996; Diekmann 1997; Dovas *et al*. 2001; Lunello *et al*. 2002; Pappu *et al*. 2005; Gieck *et al*. 2007). Tobacco rattle virus (TRV, vectored by several nematodes in the family *Trichodoridae*) also infects garlic (Diekmann 1997; ICTVdB 2006). *Iris yellow spot virus* (IYSV, vectored by thrips, *Thrips tabaci*) is an emerging problem in onion, leek and to a lesser extent garlic (Kritzman *et al*. 2001; Gent *et al*. 2006; Pappu *et al*. 2006b; Robène-Soustrade *et al*. 2006; Schwartz *et al*. 2007).

One also encounters the name *Garlic Mosaic Virus*, but there is confusion with regard to nomenclature of viruses associated with mosaic symptoms in garlic (Hanu Pappu, pers. comm.).

Numerous studies have documented yield losses due to virus infections, especially to the widely prevalent OYDV (e.g., Lot *et al*. 1998). Mixed infection (more than one virus present) is common (e.g., Fajardo *et al*. 2001; Rubies Autonell *et al*. 2005; Lunello *et al*. 2007).

In addition to the above viruses, a comparatively new genus, *Allexivirus*, in the family *Flexiviridae*, accommodates several mite-borne viruses infecting *Allium* spp. *Garlic mite-borne filamentous virus* (GarMbFV), *Garlic virus A* (Gar V-A), *Garlic virus B* (Gar V-B), *Garlic virus C* (Gar V-C), *Garlic virus D* (Gar V-D), *Garlic virus E* (Gar V-E) and *Garlic virus X* (Gar V-X) are documented (Chen *et al*. 2001; Dovas *et al*. 2001; Adams *et al*. 2004; Cafrune *et al*. 2006). New viruses continue to be detected in garlic (Mavric and Ravnikar 2005).

TISSUE CULTURE: A PRACTICAL APPROACH FOR SOME GROWERS

Garlic cloves are vegetative propagules with the consequence that viruses persist in the next generation of garlic, whether grown as seed stock or grown for the table. Most seed garlic contains viruses, although not all are conspicuously detrimental (Rosen *et al*. 2006). Chemical management of multiple virus vectors (aphids, nematodes, thrips) is difficult and expensive (e.g., Davis 1995), so alternative management strategies are desirable.

Tissue culture (usually meristem culture with one or two leaf primorida) is a technologically viable method for generating virus-free garlic. Yields and profits from virus-free clones are demonstrably greater than for infected clones (Xu *et al*. 2001). Sequential culture of meristem from shoot tips is not always successful at eradication of viruses, but improved techniques enhance success (Ayabe and Sumi 2001; Pateña *et al*. 2005), including use of primordia of inflorescence bulbils (Ebi *et al*. 2000). Analogous tissue culture techniques have been used to free vegetatively propagated *Allium cepa* var. *ascalonicum* (shallots) from virus infection (Fletcher *et al*. 1998). Cryo-preservation of garlic is effective and such cryo-preserved stocks are often virus-free (Keller *et al*. 2006), but effectiveness may vary with type (hardneck versus softneck) and tissue (bulbils versus cloves) (Volk *et al*. 2004).

Tissue culture is now used to generate disease free commercial planting stock in California (Anon 2002; Maria Jenderek, pers. comm.; Rosen *et al*. 2006), Australia (Zalcberg 2000) and planned for Canada (Fischer and Ritter 2005). To date, the technology is largely affordable only for growers producing on a large scale (Gayle Volk, pers. comm.).

RESISTANT VARIETIES

Resistance and/or tolerance are other strategies. Although

some reports are confined to possible resistance due to lack of viral symptoms or lack of detection of virus in certain cultivars (e.g., Pappu *et al*. 2006a; van Dijk 1993) other reports provide documentation on extensive testing for resistance to OYDV and LYSV in garlic (Lot *et al*. 2001).

There are also reports of resistance to fungi attacking garlic, e.g. *Alternaria porri* (Ellis) Cif. (Mehra and Batra 2005), *Fusarium oxysporum* f. sp. *cepae* (Rengwalska and Simon 1986), *Penicillium hirsutum* (Cavagnaro *et al*. 2005), *Pyrenochaeta terrestris* (H.N. Hansen) Gorenz, J.C. Walker & Larson (Rengwalska and Simon 1986), *Sclerotium cepivorum* (Nabulsi *et al*. 2001), and *Stemphylium vesicarium* (Wallr.) E. Simmons (Suheri and Price 2000). Red-skinned varieties of garlic tend to be more resistant to *Embellisia allii* than are white-skinned varieties, although some of the latter are also resistant (Dugan and Crowe, in press). However there are also reports of failure to locate resistance, e.g. for *Penicillium hirsutum* (Smalley and Hansen 1962), *Puccinia allii* F. Rudolphi (Koike *et al*. 2001), and for *S. cepivorum* (Coley-Smith and Entwistle 1988). That results of some investigations are contrary to results of other investigations on the same pathogens serves only to emphasize the complexities and difficulties of locating resistance in germplasm.

GENETICALLY MODIFIED GARLIC

It is possible to transform garlic via particle bombardment (biolistic transformation) with plasmid DNA (McGraw 1998; Park *et al*. 2002; Sawahel 2002), with obvious implications for transfer of resistance genes. Mutation via gamma radiation has been reported as generating disease resistant mutants (Al Safadi *et al*. 2000).

CHALLENGES AND OUTLOOK

Garlic production is interesting from both horticultural and sociological perspectives because of the participation of many small growers and gardeners. Garlic fairs and festivals are held annually in several cities of North America, and garlic bulbs are often traded or sold for seed as well as consumed for pleasure. These practices also occur in the UK and in Europe. The Internet has provided many further outlets for sale or exchange amongst producers with limited financial resources but abundant enthusiasm and knowledge. Although there are numerous benefits to enhanced communication and germplasm exchange amongst these enthuseasts, there are also the dangers and consequences of spreading pathogens along with the germplasm. Although most pathogens of garlic appear to be cosmopolitan, there is always the danger of introduction of pathogens or more aggressive pathogen genotypes into fields where they were previously absent.

Growers with greater financial resources are increasingly able to make use of tissue culture programs to generate disease-free planting stock, both for their own use and the market. One hopes that the formation of cooperatives or other mechanisms will allow the benefits of tissue culture to be shared with smaller producers. Refinements in diagnostic technology, especially affordable kits for virus detection, and the deployment of disease-resistance cultivars, should benefit large and small producers alike. It seems highly probable that genetically modified garlic with enhanced disease resistance or tolerance could also be beneficial, provided that such products are accepted in the market place.

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