

## **Title: DETECTION OF SYSTEMIC AND LATENT PRESENCE OF *BOTRYTIS ALLII* IN ONION TRANSPLANTS**

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### **Abstract:**

In New York, major outbreaks of Botrytis neck rot of onion, caused by *Botrytis allii* have occurred in association with the recent increases in growing onions from transplants. It has been suspected that bare-root transplants grown in Arizona could be infected with *B. allii* before they are transplanted in New York. The present study conducted during 2006 endeavored to survey onion transplants for *B. allii* contamination, to elucidate the main source of this contamination and its role in Botrytis neck rot loss in storage, and to compare the data with that developed from a preliminary and similar study conducted during 2005. In the present study which involved 50 entries (variety by grower), 81.3% of the bare-root transplants had some level of latent *B. allii*, compared to 0% for plug transplants and 0% for direct seeded seedlings. Raw and treated seed, bare-root transplants, and direct seeded seedlings, all of the same lot number, had 71.4%, 0%, 90% (of the bundles) and 0% *B. allii*, respectively. In the 2005 study which involved 35 entries, 77.8% of the bare-root transplants had some level of latent *B. allii*. The feasibilities of growing bare-root transplants free of *B. allii*, thereby preventing spread of *B. allii* from contaminated transplants, and using plug transplants free of *B. allii* as an alternative to the bare-root transplants should be considered by New York onion growers and could be utilized as procedures to reduce and hopefully eliminate the occurrence of Botrytis neck rot of onion bulbs in storage.

### **Background and Justification:**

*Botrytis allii*, the causal agent of Botrytis neck rot which is known primarily as a storage disease of onions, also has been reported to be associated with the soil-line Botrytis disease of onions (Schwartz & Mohan, 1995). Both diseases deem bulbs unmarketable. In New York, major outbreaks of Botrytis neck rot (up to 80% loss in some varieties) have occurred in association with the increase in growing onions from imported bare-root transplants over the last five years, and in particular during the very wet growing seasons of 2000 and 2004. In 2003, a serious case of *Botrytis allii* infection of onion transplants occurred in a single variety of sweet onions in several fields in western and central New York, some of which had never been cropped to onions. Just two weeks after transplanting, bare-root transplant seedlings in those fields began to rot. Gray sporulation confirmed to be *Botrytis allii* by the Cornell University Plant Disease Diagnostic Lab, occurred on the plants along the leaf sheath and oldest leaf just above the soil line. Often, 10 to 30 plants in a single row were affected while the plants in the adjacent rows were asymptomatic. Five to 17% of the plants rotted and had to be replaced with new transplants. Samples of bulbs from two of the sweet onion fields were evaluated after three months in storage and 43 and 51% had Botrytis neck rot. In adjacent fields of different varieties grown from bare-root transplants, the disease was not observed in the field, but the incidence of neck rot after three months in storage ranged from 1

to 24%. In 2004 when the incidence of neck rot was generally high, the disease occurred in the field after bulbing at low levels (< 1%) in most onion fields grown from bare-root transplants and up to 12% in sweet varieties in western New York. The disease could not be detected in direct seeded onions in the field.

### **Objectives:**

The observations noted above led to the suspicion that bare-root onion transplant seedlings can be infected latently with *Botrytis allii* before they are transplanted. Using seed and transplants that are free of disease is the first line of defense when utilizing an integrated management approach to controlling Botrytis neck rot. This research endeavored to survey onion transplants for *Botrytis allii* contamination, to elucidate the main source of this contamination, and its role in Botrytis neck rot loss in storage. Experimental design for the study was based on the experiences and results of a preliminary and similar study conducted during 2005.

### **Procedures:**

**Survey of onion transplants for *B. allii*.** Twenty nine bare-root transplant entries were collected in which 16 (6 varieties), ten (8 varieties) and three (2 varieties) were of red, yellow and sweet market classes, respectively. The majority of the bare-root transplants were imported from Arizona, but there also was an entry where the transplants were grown in sterile artificial soil in a greenhouse in New York. Eleven New York onion growers participated in the study during 2006. Identities of varieties, growers, and transplant producers were coded for anonymity of the growers and companies involved.

A bioassay technique used by du Doit *et al.* (2004) was adapted to induce sporulation of latent *B. allii*. Ten transplant bundles (~ 25 to 100 plants per bundle) per entry were randomly selected prior to transplanting as soon as possible after being imported. Each bundle was placed in a brown paper bag and stored at 32 – 40 °F for 6 to 12 weeks until the bioassay could be set up. After cold storage treatment, loose leaves and debris were shaken off, plants were rinsed under running water, dipped for 5-10 seconds in 0.5% chlorine solution, rinsed again and set on newspapers to air dry. Using a scalpel, each plant was cut in half. Plants from a single bundle then were placed in a single layer on a wire mesh in a humidity chamber. Humidity chambers consisted of plastic containers (12 qt, Sterilite®) in which a layer of galvanized hardware cloth (MAT, 5 mm mesh) was placed 1 inch over top of three moist paper towels on the bottom of the container. The humidity chambers were sealed and stored at ambient indoor temperature (no air conditioning) for one week, after which time the plants were removed and examined under a dissecting microscope for *B. allii* sporulation. Samples of suspicious *B. allii* were shipped to the Department of Plant Pathology at Cornell University in Ithaca for confirmation.

For comparison, plug transplants and direct seeded onions also were assayed for latent *B. allii*. Eight plug transplant entries were collected, of which five (3 varieties), two (2 varieties), and one entry were of the red, yellow, and sweet market classes. Two trays (200 – 244 cells per tray) per entry were randomly selected and stored under outdoor ambient temperature until the bioassays could be accomplished. Plug transplants were collected from three producers. Thirteen (red: 5 entries/3 varieties; yellow: 8 entries/8 varieties) direct seeded onion seedlings were collected by

randomly selecting ten samples of 20 plants per entry directly from the field. Seedlings were collected in June and early July when they were approximately 2 months old with 5-6 leaves, approximately the same age as when bare-root transplant seedlings are pulled or plug seedlings are transplanted. Samples were stored and assayed as previously described for the transplant onions. All direct seeded plant samples were grown in muck soil in Genesee and Orleans Counties.

**Study to elucidate source of *B. allii* transplant contamination.** To determine the source of *B. allii* contamination in onion transplants, one would need to assay *B. allii* in seed, bare-root and plug transplants, and direct seeded onions of the same seed source. With few exceptions, the varieties grown from transplants are different than those that are direct seeded. In the few instances when the same variety is grown from seed and transplants, the seed is usually from different lot numbers. Also, seed is treated and shipped directly to the transplant growers. For these reasons, it usually is impossible to make direct comparisons among *B. allii* in seed, direct seeded onions, bare-root and plug transplants, and neck rot in storage of the same lot numbers in a large scale survey. Fortunately, it was possible to compare one red (variety R, grower I) and one yellow (variety S, grower I) variety of the same lot numbers of raw and treated seed, bare-root transplants, and direct seeded plants.

## **Results:**

**Survey of onion transplants for *B. allii*.** Out of 50 entries, 81.3% of the bare-root transplants had some level of latent *B. allii*, compared to 0% of the plug transplants and 0% of the direct seeded seedlings (Table 1). Incidence of *B. allii* in bare-root transplants, as measured by the number of bundles that had some level of *B. allii*, was similar among the sweet (50.0%) and yellow (48.7%) market classes and lower in the red market class (30.5%). Incidence of *B. allii*, as measured by the percentage of plants per bundle infected, was highest in the yellow market class (5.3%), followed by the sweet (2.6%) and the red (1.9%) market classes. Sporulation of *B. allii* occurred in leaf axils and on leaf tips, but none on roots or bulbs. Notably, of all the sweet bare-root entries, the only one that did not have *B. allii* was variety P when it was grown in sterile artificial soil in a greenhouse in New York by grower N. There was much variability in incidence of *B. allii* among different entries of the same variety. An exception to this was the yellow variety K, which had 3.8 to 17.3% incidence of *B. allii* in more than 60% of the bundles in each of its three entries in two years of study (2005 data not shown).

**Study to elucidate source of *B. allii* transplant contamination.** Yellow Variety (S): seed assays yielded 71.4% and 0% *B. allii* per seed (70 seeds assayed) for raw and treated seed, respectively (Table 1). Ninety percent of the bare-root transplant bundles assayed had some level of *B. allii* (average: 12.3% per bundle; range: 3.1 to 35.3%). No *B. allii* was detected in the direct seeded seedlings (Table 1). Red Variety (R): seed assays yielded 35.7% and 0% *B. allii* per seed (70 seeds assayed) for raw and treated seed, respectively. Thirty percent of the bare-root transplant bundles assayed had some level of *B. allii* (average: 1.3%; range: 2.0 to 7.3%). No *B. allii* was detected in the direct seeded seedlings (Table 1).

## Discussion:

Results of the survey of onion transplants for latent *B. allii* clearly demonstrate that this pathogen can be introduced into an onion production system via bare-root transplant seedlings imported from Arizona. Similar results previously were obtained in 2005, where out of 35 entries, 77.8% of the bare-root transplants had some level of latent *B. allii*, compared to 0% of the plug transplants and 0% of the direct seeded seedlings. Since a transplant bundle was the experimental unit in this study, the incidence of latent *B. allii* as measured by the number of bundles per entry that had some level of *B. allii* is the most accurate measurement. It is possible that plant to plant contamination may have occurred within the humidity chambers making incidence of *B. allii* per bundle less accurate. In New York, red and sweet bare-root transplants are planted three plants per foot for an estimated 104,544 plants per acre (15 inch row spacing), yellows are planted at four plants per foot for an estimated 139,392 plants per acre. If only one plant per bundle (50 to 75 plants per bundle) was infected, this would result in 1,394 to 2,788 point sources of *B. allii* per acre, depending on plant spacing and number of plants per bundle.

It is unknown whether the inconsistency of the incidence of *B. allii* among the same varieties grown by different growers and in different years is related to the different lots of varieties as several growers were unable to provide lot numbers with their entries. A higher incidence of *B. allii* in bare-root transplants imported from Arizona in the study conducted during 2005 as compared to that in the present study may be related to the weather conditions in Arizona during transplant production. *B. allii* is a moisture-driven disease with most severe outbreaks occurring during cool and wet growing seasons (Maude and Presly 1977a, 1977b). January through March of 2005 was apparently cooler and wetter than normal (personal communication: Gary Mayfield, Sunbelt, Phoenix, AZ) and only 60 to 70% of the seed, as opposed to 80 to 90% in 2006 was returned to New York as transplants, which were generally smaller in size than normal. Alternatively, the winter of 2006 in Arizona was one of the driest on record according to the National Weather Service, Phoenix, Arizona.

The fact that *B. allii* was never detected in the present study on locally grown plug transplants or on direct seeded seedlings of similar age suggests that latent *B. allii* occurs at a considerably lower frequency, if at all, when onion seedlings are produced in these ways compared to bare-root transplant production in Arizona. How or when the bare-root transplants become infected with *B. allii* is unknown. Several factors may help to explain our findings. First, conditions for *B. allii* infection, development and spread, optimum 75 °F and 12 hours of leaf wetness (Ramsey and Lorbeer, 1986), are conceivably quite favorable during bare-root transplant production in Arizona. High density plantings (60 seeds per foot) are planted in beds that are furrow-irrigated to stimulate uniform germination. Seeds destined for bare-root transplant production are minimally treated with thiram, if anything at all. Recently, at Cornell University, J.W. Lorbeer and D.P. LoParco (personal observations) have noted the susceptibility of the emerging tip of the cotyledon of the onion seedling to infection by *B. allii*. It is also well documented that *B. allii* readily colonizes necrotic leaf tissue (Maude and Presly 1977a). Theoretically, unprotected onion seeds germinating in cool and saturated soil in the presence of *B. allii*, regardless of whether the fungus is on/in the seeds or in the soil, is an optimum condition for infection of the seedlings to occur. When the seedlings become older, outer leaves die and overlap each other in the high density plantings. Furrow irrigation at this stage could theoretically provide a moisture level to induce *B. allii* in a latent

infection to produce spores, which could readily spread from plant to plant. Additionally, *B. allii* could spread from plant to plant via contaminated knives during mowing, which typically occurs as the plants are pulled just before shipping. *B. allii* has been described as being ubiquitous in the semi-arid onion growing region of central Washington (du Toit *et al.* 2004). However, in the temperate climates of the United Kingdom (Maude and Presley, 1977a, 1977b, 1982) and New York (J.W. Lorbeer – personal observations), *B. allii* is not considered ubiquitous and major outbreaks of Botrytis neck rot have been traced to infected/infested seed. In the case of background sources of *B. allii*, this could explain the higher incidence of infected bare-root transplant seedlings grown in Arizona compared to the locally grown direct seeded seedlings. Plug transplants, on the other hand, are grown in a sterile artificial soil and the seed used to grow them is fungicide treated to the same extent as seed destined for direct-seeded onions. In the 2005 study, *B. allii* was not detected in sweet plug transplant seedlings of the same lot number as bare-root transplants where *B. allii* was detected.

The study, in which we assayed for latent *B. allii* in raw and treated seed along with the bare-root transplants and direct seeded seedlings from the same lot number of two varieties, showed two things. First, the seed lot that had a higher incidence of *B. allii* (variety S, grower I) also had higher percentages of bundles with some level of *B. allii* and average *B. allii* per bundle. Incidence of Botrytis neck rot in storage also tends to increase as incidence of seed infection/infestation increases (Maude and Presley 1977b). Second, *B. allii* was not detected on treated seed, nor was it detected in the direct seeded seedlings which were grown from the same treated seed, unlike the bare-root transplants. Initially, such results indicate that proper fungicide seed treatments may eliminate *B. allii* from the seed. However, they could also mean that the seed treatment only prevented isolation of the fungus from the seed under the conditions of the seed test. *B. allii* could have survived the fungicide treatment and been capable of infecting the seedlings. Alternatively, the fungicide treatment could have killed the fungus colonizing the seed and the germinated seedlings would presumably be free of *B. allii*. The efficacy of fungicide seed treatments to control *B. allii* infection of onion seedlings warrants further investigation.

Ultimately, the extent to which latent *B. allii* in bare-root transplant seedlings translates into Botrytis neck rot in storage depends on how favorable the weather is during the onion growing season for development and spread of *B. allii*. During the extremely hot and relatively dry growing season of 2005, latent *B. allii* in bare-root transplants generally did not result in proportionate levels of Botrytis neck rot in storage of the red onions that are considered to be storage onions (data not shown). Alternatively, the sweet onions had over 50% Botrytis neck rot out of storage. However, sweet onions are not considered storage onions, and would not normally be stored into January. Similarly, the yellow varieties grown from transplants are also not typically storage varieties and are usually sold shortly after harvest. Thus, even if there was a 1:1 relationship between latent *B. allii* in transplants and Botrytis neck rot in storage, this should not be of economic concern for transplant varieties that are not being stored for the longer term, unless they develop infection by *B. allii* in the field resulting in unmarketable bulbs, or if they spread inoculum and infect onion plants in nearby fields where the bulbs are destined for long-term storage. In cool wet years, the potential for development and spread of *B. allii* from latently infected transplants may result in economic losses.

In conclusion, bare-root transplants from Arizona are indeed being imported into New York already contaminated with the *B. allii*, and this fact may at least in part explain the recent increase

in outbreaks of *Botrytis* neck rot in New York. The feasibilities of growing bare-root transplants free of *B. allii* in Arizona, preventing secondary spread of *B. allii* from contaminated transplants, and using locally produced plug transplants that are free of *B. allii* as an alternative, warrant further investigation.

**Project Location(s):** Western and Central New York; Ithaca, New York

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