"Final Project Report to the NYS IPM Program, Agricultural IPM 2002-2003"

Title: Nature and Control of the Aspergillus Black Mold Disease of Onions in New York

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Type of grant: Biological control and pest biology

Project location: The findings of the study could be applied in all areas of New York as well as
nationally and internationally wherever onions are grown.

Abstract: Onion seedlings became infected with *Aspergillus niger* either when seed harboring the fungus was planted in soil free of the fungus or when seed not harboring *A. niger* was planted in soil artificially infested with the fungus. *A. niger* was detected in the roots, basal plates, cotyledons, and leaves of the infected seedlings. Onion plants grown from seedlings infected with *A. niger* from both seedborne and soilborne sources were detected to harbor the fungus in a symptomless manner in tissues of the plants until they became mature. Either prior to harvest, at harvest, or after harvest the plants and/or resulting bulbs exhibited symptoms of black mold. Physiological changes in the plants as they mature and or environmental conditions such as high temperatures and moisture levels at maturity most likely can regulate the change of *A. niger* from the endophytic symptomless nature to the production of black mold symptoms. Propagule levels of *A. niger* in fields (organic soil) cropped to onions differed substantially between fields, perhaps due to different cultural procedures and/or cropping patterns. These differences along with the use of seed either free of *A. niger* or contaminated with the fungus could lead to divergent levels of black mold in onions grown on the different fields. Studies involving cooling and drying of onion bulbs to prevent expression of black mold symptoms as well as temperature regulation of onion seedling infection by soilborne inoculum of *A. niger* in miniculture studies are still to be finalized.

Background and justification: New York onion growers, especially those in Orange County, continue to request that research leading to an effective control of black mold caused by *A. niger* be conducted. The potential for the occurrence of the disease each year, the economic losses associated with past occurrences of the disease, and the desire to obviate future severe outbreaks of the disease strongly drive the continuing request of the growers that a strong research program on the disease be maintained. It is desirable to control black mold in an IPM mode and hopefully avoid the need for the application of fungicide sprays late in the growing season as strongly advocated by some New York onion growers who believe that such sprays might reduce or eliminate subsequent occurrences of black mold. Such late season sprays frequently are not needed for the control of onion leaf diseases such as Botrytis leaf blight and Alternaria purple blotch as indicated by IPM field scouting reports and BLIGHT-ALERT forecasts. Therefore, it is important, if possible, that non-fungicide procedures be identified

that will reduce or eliminate black mold so that the growers will not feel compelled to attempt to control the disease with late season fungicide sprays. Control of black mold by non-fungicide procedures developed from that knowledge base and focused on drying and cooling of onions after harvest also would limit the potential for unneeded applications of fungicides which can affect the quality of ground water and the environment within the onion cropping system. Also, recent research has suggested that *A. niger* colonizes the soils of different fields at different levels, and thus it appears population levels of *A. niger* in the soils may be regulated by specific cultural and cropping patterns.

Cornell IPM sponsored research conduted on *A. niger* and black mold has indicated: (a) the nature of onion flower infection by *A. niger* leading to the infestation of onion seed by the fungus; (b) the potential importance of seedborne inoculum of *A. niger* in the subsequent occurrence of black mold; (c) the systemic nonsymptomatic colonization of onion seedlings and young plants by *A. niger* from seedborne and soilborne sources of inoculum; (d) the identification and successful testing of a selective medium (PLYA) for determining propagule levels of *A. niger* in New York organic soils cropped to onion. Preliminary studies have been developed data indicating considerable differences in soil population levels of *A. niger* in selected onion fields. Additional preliminary research, and which needs to be verified, has suggested that onion seedlings infected by seedborne or soilborne sources of *A. niger* can symptomlessly harbor the fungus until the plant matures and the bulbs are harvested and then causes black mold to occur when environmental conditions (hot and moist) favoring the disease occur.

Objectives: The objectives of the research were: (1) to determine the parameters of drying and cooling procedures applied to harvested onions necessary to prevent the development of black mold on onions known to harbor the presence of *A. niger* (not yet completed), (2) to quantify the comparative populations of *A. niger* in the soils of selected fields cropped to onions which have had histories of differing levels of black mold in past years and relate the soil population levels of *A. niger* and cultural practices at harvest time to occurrence of black mold in onions produced on the selected fields; (3) to identify crops or other plant species which regulate (increase, maintain, or decrease) the population levels of *A. niger* in New York organic soils cropped to onion by using a miniculture procedure recently developed and successfully tested for this purpose (this objective has not been possible to achieve during the first 8 months of the project); (4) to determine under controlled environmental conditions the dynamics of the infection process leading to the symptomless presence of *A. niger* in onion seedlings, young plants, and maturing onions resulting in the occurrence of black mold in mature onion plants and bulbs.

Procedures:

Systemic Infection Study: Prior to April 1, 2002 autoclaved soil (Cornell potting soil) was infested artificially with *A. niger* (isolate I-S). The fungus was cultured on potato dextrose agar (PDA) for two weeks at 30°C and two heavily sporulating cultures (including PDA) and 500 ml of sterile, distilled water were mixed in a blender at low speed for one minute. The slurry was mixed by hand with 2000 g of autoclaved soil until the soil appeared uniformly moist. The infested soil was placed into two sterilized flats and thirty-five onion seeds were sown in each flat. These two flats were labeled T-1. For the experimental control, 2000 g of the autoclaved soil (Cornell potting soil) was mixed with 500 ml of sterile distilled water, placed into sterilized flats, and seeded. These two flats were labeled T-2.

Sixteen days after the seed had germinated and young seedlings had developed, a bioassay was conducted to determine if *A. niger* was present in different tissues of the seedlings. Ten seedlings from T-1 and T-2 were selected arbitrarily and harvested by hand to keep the root

systems relatively intact. The seedlings were rinsed briefly with distilled water to remove debris from the roots. Five seedlings apiece from T-1 and T-2 were rinsed in agitated water for ten minutes and then dried on sterile paper in a laminar flow hood. Each seedling was dissected into root, basal plate, lower leaf, and leaf tip sections. Tissue sections from each seedling were grouped together according to section type and plated on acidified potato dextrose agar (APDA). The plates were sealed and placed in a 30ºC incubator for 72 hours. The remaining seedlings received the same treatment as described, plus two additional steps. After the seedlings were rinsed in agitated water for ten minutes, the seedlings were surface disinfested for three minutes in 0.525% sodium hypochlorite and then rinsed three times with distilled water. The seedlings were dried on sterile paper in a laminar flow hood and dissected. The tissue sections were plated on APDA and incubated at 30ºC. After 72 hours, the plates were inspected visually for *A. niger*.

T-1 and T-2 seedlings were transplanted individually 16 days after they germinated into sterilized clay pots containing autoclaved Cornell potting soil. Ten months after germination, ten T-1 plants were assayed as described previously. The remaining T-1 and T-2 onion plants were assayed and evaluated for *A. niger* 349 days after they had germinated. The plants with their attached bulbs were harvested by hand and then dried on the greenhouse bench for 27 days to ensure that the inside tissues of the bulb necks were dry. T-1 and T-2 plants then were topped with sterile shears and placed in sterile humidity chambers. The bulbs were incubated for twelve days at either 30ºC or 33.3ºC. After incubation, the bulbs were visually inspected and evaluated for the presence of *A. niger* and occurrence of black mold symptoms.

Soil Assay Study: The evaluation of the field samples for determining the colony forming units (cfu's) of *A. niger* involved weighing three, 1 g soil samples from every field sample collected. Each 1 g sample was suspended in 9.5 ml of sterile, distilled water, and was agitated vigorously for five minutes. The soil suspensions were then allowed to settle for at least one minute. Ten-fold dilutions were prepared by adding 0.1 ml of the soil suspensions to test tubes containing 9.9 ml of sterilized, distilled water. The final dilution value of 10^-4 was achieved by dispensing 1 ml of the soil suspensions to a petri dish containing Acidified-Prune-Lactose-Yeast Agar. Three ml of the soil suspension was dispensed from every test tube per 1 g of soil. The soil suspension was evenly distributed over the agar surface by using spread plate techniques. The soil suspension was allowed to settle on the agar surface for at least one minute before each plate was sealed with Parafilm. The plates were placed in an inverted position in a 30 C incubator for 72-hours. After a 72-hour incubation period, the plates were examined and the colonies of *A. niger* were counted. One g of moist soil from each field sample was dried in an oven to determine the dry weight of each field sample. The dry weight of each field sample was used to calculate the cfu's of *A. niger* per gram of dry soil.

Results and discussion: *A. niger* was isolated from T-1 but not from the T-2 experimental control seedlings in the assay made 16 days after germination. T-1 (rinse only) seedlings had a recovery rate of 100% from all tissue sections. T-1 (rinse and surface disinfested) seedlings had recovery rates of 80, 60, 60, and 40% from the root, basal plates, lower leaf, and leaf tip sections, respectively. No symptoms of disease or signs of *A. niger* were visible in the T-1 plants when the assay was conducted. *A. niger* was not recovered from T-2 seedling tissues, nor detected externally on T-2 seedlings. In the assay performed ten months after the plants had germinated, only T-1 plants were evaluated for *A. niger*. T-1 (rinse only) plants had recovery rates of 100, 100, 80, and 100% from the root, basal plate, lower leaf, and leaf tip sections, respectively. T-1 (rinse and surface disinfested) plants had recovery rates of 100, 40, 100, and 100%. Black mold was visible beneath the scales in the neck, shoulder, and flank regions in four out of the ten onion bulbs harvested for this assay.

When the T-1 and T-2 onion plants were harvested 349 days after germination, some of the plants in both groups exhibited sporulation of *A. niger* on surface areas of the plants and bulbs and this sporulation was still evident after the plants had dried for 27 days on the greenhouse bench. After topping the T-1 and T-2 onion plants and placing the bulbs in the humidity chambers at either 30°C or 33.3°C, both groups had some degree of external and/or internal *A. niger* sporulation. T-1 bulbs incubated at 30ºC had heavy internal sporulation of *A. niger* inside the necks and beneath the scales of the shoulder and flank regions typical of symptoms of black mold. The surfaces of the bulbs also were covered with fluffy, white mycelium. T-1 bulbs incubated at 33.3ºC exhibited the greatest degree of external sporulation. Black conidia of *A. niger* covered the exterior scales of the bulbs and also were present in the neck tissues. Externally, the bases of the bulbs also were covered with fluffy, white mycelium that appeared to emerge from the basal plates of the bulbs. T-2 bulbs incubated at 33.3ºC exhibited greater degrees of internal and external sporulation than T-2 at 30ºC, but much less than T-1 bulbs at 30ºC and 33.3ºC. Some black conidia were visible in the neck tissues of the bulbs, while externally there were some conidia on the bases of the bulbs. Since the seedlings of the T-2 plants had not been detected to harbor *A. niger* internally, it is suspected the limited presence of *A. niger* in the T-2 bulbs resulted from an airborne source of the fungus in the greenhouse.

After incubation in humidity chambers at either 30ºC or 33.3ºC, T-1 bulbs had a greater degree of external and/or internal sporulation of *A. niger* than did the control group, T-2. The difference in degree of sporulation may be explained by the different nature of infection in T-1 and T-2. T-1 bulbs appeared to have systemic infections that were initiated by inoculum in the soil. In contrast, T-2 bulbs appeared to have superficial infections caused by airborne propagules of *A. niger* present in the greenhouse. An onion in which the fungus has colonized internal tissue of the bulb such as the basal plate would likely be more severely affected than an onion with some fungal propagules scattered only on the exterior bulb surface. Incubation of the bulbs at 33.3ºC was more conducive to sporulation of *A. niger* than was incubation at 30ºC. At 33.3ºC, both T-1 and T-2 bulbs had a greater degree of black mold than either group incubated at the lower temperature. The temperature range of 28-34ºC is considered optimum for growth of *A. niger*.

The data generated by this study suggest that *A. niger* can function as an endophytic fungus in onion plants. The fungus may remain latent in onion tissue until the plants become mature and/or favorable environmental conditions trigger the expression of the pathogen. In the assays conducted at sixteen days and at ten months after germination, *A. niger* was isolated at high rates from surface disinfested tissues. This is significant for three reasons: (a) consistent recovery of the fungus from tissues that were surface disinfested for three minutes with 0.525% sodium hypochlorite is indicative of an internal infection by the fungus; (b) the recovery of the fungus from the roots, basal plates, lower leaves, and leaf tips demonstrates the systemic presence of the fungus throughout the seedlings; (c) the fact that the fungus was isolated from the onion plants in assays conducted soon after germination, during growth of the plants, prior to harvest, and after harvest, as well as from the onion bulbs after a heat and high moisture treatment, is further evidence of the endophytic nature of *A. niger*. The data from this investigation as well as those made previously (1, 2) indicate that *A. niger* can enter onion plants via soil containing the fungus and by seedborne inoculum of the fungus and can result in the fungus becoming endophytic in the plant throughout its growth and bulb formation. The transition from a latent stage to active infection appears to occur when the onion plants reach maturity and/or when the resulting bulbs are subject to high humidity and temperature.

The levels of *A. niger* in the four New York fields (organic soil) cropped to onion during 2002 differed substantially among the fields (between 0 to 15,000 propagules of *A. niger* per gram of dried soil) perhaps due to differences in cropping patterns or other cultural practices (Table 1). Levels of *A. niger* in soils sampled during 2001 ranged from 1,000 to 14,000 propagules per gram of dried soil. These differences possibly could lead to different occurrence levels of black

mold on onions grown on the different fields and within the same field. During 2002 fields 1-3 with the highest overall levels of soilborne *A. niger* were observed to have many onions at harvest exhibiting black mold symptoms.

It now appears that New York onion growers can minimize the occurrence of black mold by using onion seed free of contamination by *A. niger*. Procedures for providing seed not contaminated with the fungus need to be developed. The biology of late season infection of onion plants by airborne and soilborne inoculum needs clarification. However, it now appears that seedborne inoculum is the factor leading to some of the most serious losses due to black mold that growers have encountered and explains the occurrence of the typical disease symptoms on the sides of onion bulbs under the outer scales. Also, it was observed during a prolonged host period that in one field with a history of black mold when the onion were harvested during the coolest parts of the day and handled gently the incidence of black mold was greatly reduced as compared to onions harvested from the same field during the warmest part of the day and not handled as gently. This indicates that the timing and quality of the harvest procedure may be an important factor in reducing the incidence of black mold.

References:

1. Ransom, V. E. 2000. Investigation of potential inoculum sources of *Aspergillus niger*, the cause of black mold of onion. Department of Plant Pathology, Cornell University, Ithaca, NY, MPS Project Report. 89 pp.
2. Sirois, K. L., LoParco, D. P., and Lorbeer, J. W. 1998. Development of a bioassay to determine the presence of specified fungal pathogens of onion. pp. 353-360. In: Proceedings of the 1998 National Onion (and other Allium) Research Conference. Sacramento, California, Dec. 10-12, 1998.9

Table 1. Mean colony forming units of *Aspergillus niger* recovered from organic soils of Orange County, New York cropped to onions

	July	August	September
Field 1 Location #1	11.320 x 10^3	6.274 x 10^3	1.413 x 10^3
#2	1.380 x 10^3	1.219 x 10^3	2.499 x 10^3
#3	1.349 x 10^3	7.906 x 10^3	7.356 x 10^3
#4	2.888 x 10^3	9.166 x 10^3	13.371 x 10^3
#5	8.501 x 10^3	8.412 x 10^3	1.921 x 10^3
Field 2 Location #1	4.856 x 10^3	6.277 x 10^3	6.255 x 10^3
#2	4.520 x 10^3	7.463 x 10^3	15.027 x 10^3
#3	3.631 x 10^3	6.913 x 10^3	2.257 x 10^3
#4	5.421 x 10^3	7.435 x 10^3	3.372 x 10^3
#5	2.455 x 10^3	5.448 x 10^3	11.903 x 10^3
Field 3 Location #1	8.501 x 10^3	4.623 x 10^3	3.636 x 10^3
#2	10.703 x 10^3	11.533 x 10^3	7.827 x 10^3
#3	3.958 x 10^3	5.000 x 10^3	12.345 x 10^3
#4	5.512 x 10^3	5.748 x 10^3	15.308 x 10^3
#5	7.882 x 10^3	6.524 x 10^3	8.955 x 10^3
Field 4 Transect A (10′)[a]	1.863 x 10^3	1.768 x 10^3	0.270 x 10^3
(35′) [a]	0.951 x 10^3	0.476 x 10^3	1.211 x 10^3
(60′) [a]	0.800 x 10^3	1.000 x 10^3	4.057 x 10^3
(85′) [a]	0.329 x 10^3	0.500 x 10^3	2.357 x 10^3
Field 4 Transect B (10′) [a]	0.791 x 10^3	1.895 x 10^3	1.883 x 10^3
(35′) [a]	1.474 x 10^3	0.192 x 10^3	0.0
(60′) [a]	0.361 x 10^3	0.500 x 10^3	0.0
(85′) [a]	0.850 x 10^3	0.0	0.0

[a]10′→85′ are samples made along a transect in one bed of onions at the distances indicated from the edge of the field.