Nature and Source of Inoculum of *Aspergillus niger* Causing the Aspergillus Black Mold Disease of Onions in New York

Research Report for 2000

New York State Integrated Pest Management Grants Program

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Introduction: Onion growers in New York especially those in Orange County continue to remain quite concerned about the possibility that a serious outbreak of Aspergillus black mold caused by *Aspergillus niger* could occur in any given year, particularly if weather favoring the disease (hot, humid, and rainy conditions) occurs during the latter part of the growing season (mid July to the end of August). A major outbreak of the disease occurred in Orange County in 1995 with serious economic consequences for a number of growers and the disease has been present each year since 1995 generally at low levels although a number of minor outbreaks since then have occurred. Since knowledge concerning the etiology of the disease is limited, continued research on the nature of *A. niger* as an onion pathogen is required to ultimately develop effective management procedures based on that knowledge to prevent both major and minor outbreaks of the disease as well as to attempt to prevent the low levels of the disease that occur annually on most New York onion farms. New York onion growers continue to request that in depth research on the nature and control of black mold be continued each year until reliable management procedures for control of the disease are developed.

Studies continued during 2000 on the nature of onion seed surface infestation and internal infection of onion seed by *A. niger* and systemic infection of onion seedlings by the pathogen from seed and soil sources. A number of selective media were tested for quantifying *A. niger* in organic soils cropped to onion. Prune Lactose Yeast Agar (PLYA) was selected for utilization in studies for relating soil inoculum levels and cultural procedures in selected fields at commercial onion farms in New York to occurrence of black mold in onions grown on those fields. PLYA also was utilized for detecting airborne propagules of *A. niger* in onion fields at harvest time. Although the study initially proposed was broad in scope, the limited support provided by the IPM program necessitated reducing the breadth of the research to: (1) the nature of onion seed, seedling, and young plant infection by *A. niger*; (2) the identification of the selective medium for quantifying the levels of *A. niger* in organic soils cropped to onion as well as for detecting airborne propagules of the fungus in onion fields at harvest time; and (3) to a cooperative study with IPM personnel (John Mishanec and Teresa Rusinek) on the effects of lifting, undercutting, and windrowing onion plants prior to harvest and the subsequent occurrence of black mold as related to soil population levels of the

fungus in the field areas where the onion plants were grown, lifted or undercut, and windrowed.

Objective: The research was conducted to determine the nature and source of inoculum of *A. niger* as related to the methods of infection by the pathogen on onion seed stalks and flower parts leading to surface infestation/internal infection of onion seed and the subsequent infection of onion seedlings and ultimately of mature plants. The research as initially planned was to determine infection pathways if any of *A. niger* on onion plants and on onion bulbs late in the growing season immediately prior to harvest, during harvest, and in storage. Because of reduced funding the research conducted was limited to onion flower and seed infection, infection of onion seedlings and young plants by seedborne and soilborne inoculum, identification and use of a selective medium for soil population studies of *A. niger* in organic soils cropped to onion, and a cooperative study with IPM personnel relating soil population levels of *A. niger* to lifting, undercutting, and windrowing field grown onions to subsequent levels of black mold.

Procedures And Results: Onion flowers were inoculated with *A. niger* at six different stages of development in order to assay the seeds produced for the presence of the fungus. This was the third consecutive year that this experiment has been conducted. The data collected from the study indicated that *A. niger* was present on seeds in all treatments, and that the levels varied greatly depending on the flowing stage at which inoculation occurred. The seeds in the 2000 study became infested only at the surface level. In the experiments conducted during 1998-99 onion seed also was internally infected. This suggests that climatic conditions and other factors may affect the nature of the location of *A. niger* on onion seed (surface or internal). The results also suggest that when onion seed is infested by *A. niger* solely on the surface of the seed, a fungicide seed treatment might be used to control future growth of the fungus. Onion seeds in the 2000 study appeared to be most susceptible to infestation by *A. niger* from the period after the sheath had unfolded from the umbel and the florets were still closed until capsule formation. This result was in accordance with those of the experiments conducted during 1998-99.

A bioassay of onion seedlings was accomplished to determine if *A. niger* could infect young onion plants through the roots and become endophytic. Onion seedlings were grown from seeds inoculated with spores of *A. niger* previous to sowing. Onion seedlings also were grown from clean seed sown in soil infested with *A. niger*. The fungus was isolated from seedlings and young plants grown from inoculated seed, particularly from the roots, basal plates, lower leaves, and occasionally from leaf tips. A number of the plants from this experiment are continuing to be grown in a glasshouse at Ithaca to maturity and will be sampled several times throughout that growth period to determine if *A. niger* is still present systemically in the plants. *A. niger* was not isolated from any tissues of seedlings grown in infested soil. In a previous experiment conducted during 1998, seedlings grown in soil infested with *A. niger* became infected by the fungus. It is possible that at the soil inoculum levels utilized during 2000,

insufficient number of propagules of the pathogen were present in the soil for infection to occur. Future experiments should clarify the reason(s) for the different results.

Seeds of seven different onion varieties were assayed for the presence of *A. niger.* The seeds were from seed lots used in a variety trial conducted in Orange County during 1999 in which several varieties in the trial had high levels of black mold in storage. The purpose was to determine whether there was a connection between seeds infected with *A. niger* and the incidence of black mold on the mature onions in storage of different varieties. The results indicated that *A. niger* was present on only a low percentage of the seeds for all the varieties.

To study soil population levels of *A. niger*, three selective media previously utilized in other studies were evaluated for their usefulness in detecting *A. niger* in soil from onion fields in Orange County. Sorbose-Based Media (SBM), Rose Bengal Agar (RBA), and Prune Lactose Yeast Agar (PLYA) were tested, as well as Potato Dextrose Agar (PDA) as a comparison. PLYA appeared to be the best suited for extracting and identifying *A. niger* from the organic soils of Orange County cropped to onion. PLYA then was used to assay inoculum levels of *A. niger* in organic soil from six different onion fields in Orange County. These fields were chosen based on their history of black mold. Two of the fields historically had yielded onions that had developed black mold in the field an in storage while four of the fields were reported to not have a history of black mold. Soil samples were taken in May, June, and July during the 2000 onion growing season and the number of colony forming units per gram of soil were determined and compared between the fields. The results showed that it was possible to quantify levels of *A. niger* in organic soils cropped to onion in Orange County using PLYA. Propagule numbers of *A. niger* in the soils sampled increased from May to July. However, no correlation in this study was found between soil population levels of *A. niger* and the history of black mold occurrence for the fields studied. Additional studies following this approach could indicate that a relationship does exist between *A. niger* soil populations and the occurrence of black mold.

In a second soil population study of *A. niger* conducted during 2000, soil samples were collected from seven onion fields of three grower cooperators in Orange County during September and assayed for *A. niger* populations. The numbers of Colony Forming Units (CFU) or propagules of *A*. *niger* per gram of soil (dry weight) in six of the seven soils were 0.3, 0.3, 0.6, 1.0, 2.8, and 3.9 X 10^3 CFU (Table 1). *A. niger* was not detected in the soil of the seventh field. Onion samples were collected at harvest time from the seven fields, stored in Ithaca, and were examined for the incidence of black mold on December 13 (Table 1). Each field consisted of side-by-side plots in which the same variety was planted at the same time and grown to maturity. The varieties used were New York Early, Uniglobe, Duration, Sabroso, Criterion, and Barrage. For each of the side-by-side plots in each field, the grower cooperator left the onions in place in one of the plots and in the other plot either lifted or undercut the onions several days before the onion plants were sampled ($\pm$ 50 plants) from areas in the beds that were approximately 30 feet long by 10 feet wide. The plants were topped when sampled and the bulbs placed in storage until December 13 when they were examined for black mold

and other storage diseases. The soil samples for quantifying the levels of soilborne *A. niger* propagules were taken from the same areas in each bed from which the onions were harvested. The data derived from this experiment are presented in Table 1. Only two onions were detected with black mold at the end of the storage period.

Discussion: The results from the study on the susceptibility of onion seeds to infection by *A. niger* when onion flowers were inoculated with the fungus at various stages of development closely resemble those obtained in an earlier study in 1998. *A. niger* appeared to be capable of infecting seed through the flowers from the period after the sheath had unfolded from the umbel and the florets were still closed until capsule formation. Since in the present study the seeds were infested only on the surface rather than under the seed coat, this may indicate that depending on the location of *A. niger* on the seed, treatments with fungicides prior to planting may be helpful in reducing the amount of onion seedling infection by *A. niger*. Measures that prevent onion flowers from becoming exposed to *A. niger* during the stages when seed are most susceptible to infection also might reduce the incidence of *A. niger* on onion seed. Whether seed infestation or infection by *A. niger* can result in subsequent infections of the mature onion bulbs by the pathogen remains to be determined.

A. niger could be detected in most seedling parts grown from seeds inoculated with the fungus prior to sowing. This indicates that onion seedlings can become infected from seeds carrying *A. niger* and that the fungus may be capable of becoming endophytic in the seeding. Whether the fungus gains ingress as the seed germinates or through the roots or other plant parts after germination remains to be determined.

When seeds from seed lots of the seven varieties from the 1999 variety trial which had differing levels of black mold in storage were assayed, all the seeds exhibited only a low infection with *A. niger*. This suggests, while seeds may be an important source of inoculum, other sources such as soil and air may be important as well. Additional factors such as weather conditions during the growing season and at harvest along with storage conditions also should be taken into account when determining the risk of infection by *A. niger* on onions.

Three selective media previously utilized in other studies were evaluated for their suitability for studying soil population levels of *A. niger* in fields cropped to onions in Orange County, New York. The evaluations showed that Prune Lactose Yeast Agar (PLYA) could be used effectively for detecting *A. niger* in organic soils using the soil dilution method. Rose Bengal Agar (RBA) modified with Ronilan (a fungicide) also was effective in detecting *A. niger* in organic soil.

In the six fields cropped to onion in Orange County, that were tested for levels of *A. niger* at three different times in the growing season (two fields had a history of producing onions that developed black mold in storage while the other four did not have such a history), the population levels increased significantly from May to July when the onions were bulbing. The rise in population levels of *A. niger* in those soils may have coincided with growth stages of the onions and could have been affected by weather

conditions and/or management practices. In this study, however, no correlation was found between soil population levels of *A. niger* and fields with a history of black mold.

In the cooperative trial with the IPM personnel involving onion field drying practices (lifting, undercutting, and windrowing), only two onions were detected with black mold after storage. Black mold levels at Orange County onion farms were very low during 2000 due to the rather mild cool weather that is unfavorable for the occurrence of black mold and which occurred during the growing season as well as at the time of harvest. However, it is to be noted that the field in which one of the onions with black mold was grown, this field was the only one of the seven in the trial in which black mold was observed both on onion plants throughout the field (inside and outside the trial areas) prior to harvest and in onions after storage. This field also had the highest level of *A. niger* in the soil (3.9×10^3 CFU or propagules) of the soils sampled. The results of the study suggest that PLYA can be a useful and reliable medium on which to assay population levels of *A. niger* in organic soils cropped to onion in New York. Although the medium was effective for this purpose in the drying trial conducted, it is planned to continue to experiment in the laboratory to develop an even more effective medium for assaying soil samples for *A. niger* as well as for use in air sampling for *A. niger*. PLYA was able to detect extremely low levels of *A. niger* in the air over onion fields at the time of harvest during 2000 but the rapid growth of other airborne fungi on the PLYA plates often obscured what appeared to be *A. niger* colonies. It should be possible to modify the medium so that it's utility in sampling for airborne propagules of *A. niger* will be improved. However, from the results of the soil sampling and observations in the air sampling made during 2000, it presently appears that soilborne propagule levels of *A. niger* are relatively much greater than airborne propagule levels of the pathogen and that soil sampling for the fungus may be a viable method for predicting the possibility of outbreaks of the disease on onions grown on individual fields. Also, it was observed that *A. niger* populations differed somewhat or greatly among soils cropped to onion and that the soils sampled during 2000 with the lowest levels of *A. niger* were soils that had been rotated to other crops in the past. This suggests that crop rotation could be a viable approach to controlling black mold in future years when environmental conditions favor outbreaks of the disease.

The findings in 2000 provide a clearer understanding of the biology of *A. niger*. However, they also raise important questions. Though onion seeds, seedlings, and young plants can become systemically infected with *A. niger*, how important is this to the subsequent occurrence of black mold on mature onion plants just prior to harvest and onion bulbs in storage? What is the effect of different soil population levels of *A. niger* under favorable environmental conditions for the pathogen on black mold occurrence at harvest time and in storage? Continued aggressive fundamental and applied research in a combined mode from an IPM approach is needed to increase the knowledge of *A. niger* as a pathogen of onions and improve the possibilities for the control of black mold.

Table 1. Onion Field Drying Practices (Lifting, Undercutting And/Or Windrowing) as Related To Soil Populations Of *Aspergillus niger* And Subsequent Levels Of Black Mold In Storage.

Grower	Variety	FDP[a]	T[b]	H[b]	BM[b]	BD[b]	BS[b]	PEN[b]	CFU[b]
1	NY Early	NL	48	38	0	6	4	--	3.9×10^3
1	NY Early	U	46	35	1	7	3	--	3.9×10^3
1	Uniglobe	NL	48	46	0	2	0	--	1.0×10^3
2	Duration	L	49	46	1	1	1	--[c]	0.6×10^3
2	Duration	NL	43	34	0	5	4	--[d]	0.6×10^3
2	Sabroso	L	51	46	0	3	2	--[e]	0.3×10^3
2	Sabroso	NL	51	44	0	5	2	--	0.3×10^3
2	Criterion	L	50	48	0	1	1	--	0.3×10^3
2	Criterion	NL	51	50	0	1	0	--	0.3×10^3
2	Duration	L	48	47	0	1	0	--	0.0×10^3
2	Duration	NL	55	54	0	1	0	--	0.0×10^3
3	Barrage	NL	53	53	0	0	0	--	2.8×10^3
3	Barrage	L(W)	51	42	0	0	9	--	2.8×10^3

[a]Field Drying Practices (FDP): NL = Onion Plants Not Lifted Or Undercut; L = Onion Plants Lifted, Run Over Chain, And Placed On Surface Of Soil; U = Undercut With Blade And Onion Plants Left In Place; W = Onion Plants Placed In Windrows After Lifting.

[b]Evaluation Status Of Onions After Storage: T = Total Onions; H = Healthy; BM = Black Mold; BD = Bacterial Decay; BS = Black Spots On The Outer Scales Of Onion Bulbs; PEN = Penicillum Infection; CFU = Colony Forming Units/Of *Aspergillus niger* Per Gram Of Air Dried Soil. CFU Also = Number Of Propagules.

[c]Light Incidence Of Penicillium Infection.

[d]High Incidence Of Penicillium Infection.

[e]Moderate Incidence Of Penicillium Infection.