Effects of nitrgen source and rate on the growth of the sesame-wilt fungus, *Fusarium oxysporum* f. *vasinfectum* (Atk). Snyder et Hansen.

by Jong Seong Park

Summary

The present study was undertaken to investigate the effects of different nitrogen source and rate on the growth of *Fusarium oxysporum* f. *vasinfectum* which is known to be a noticeable fungus causing the wilt disease of both sesame and cotton in Korea.

From the results of this study, it was known that different N source and rate markedly affect the growth of *Fusarium oxysporum* f. *vasinfectum* Among four N sourses were used in this study, nitrate-N and urea-N were appropriate N source for the growth of fungus. Above all, nitrate N was the best N source because it is utillzed in more extensive range of concentration in comparison with the other N source by the fungus. On the other hand, ammonia-N is of littie avail for the growth of the fungus because of the formation of unusual colonies with wavy margin and bead-like mycelial cells in addition to marked reduction of mycelial growth and sporulation of the fungus irrespective of concentration. Judging from the formation of such an abnormal colony and bead-like mycelial cell which is known to be a characteristic of "staling-type" growth of fungi, the effect of ammonia-N on the growth of *Fusarium oxysporum* f. *vasinfectum* is similar to that of phenoxy compounds on some other fungi previously investigated by some workers. Ammonium and nitrate N also was not considered to be an appropriate N source for the growth of the fungus because of the formation of colonies with slight wavy margin and appreciable reduction of mycelial growth and sporulation in higher concentration than 50meq., although much or less masking of the irregularity of colony occurs. Therefore, ammonia N alone or any other N combined with ammonia N is of little avail for the growth of *Fusarium oxysporum* f. *vasinfectum*.

Introduction

Fusarium oxysporum f. *lycopersici* (4, 10) and *Gibberella fujikuroi*(11) in a solution of apropriate pH containing ammonium nitrate, utilize mainly the nitrate-N in the first period of growth lasting some 2-3 weeks and later also utilize the ammonia-N. Nose (7) indicated that *Fusarium falcatum* pathogenic on cotton and sesame showed unusual growth on the culture medium containing ammonia sulfate alone as nitrogen source. Presley (9) and Jhonson(3) reported that the source and rate of nitrogen markedly affected the growth of *Verticillium albo-atrum* which is also known to be a wilt pathogen.

These results suggest that different N source and rate may influence, or be involved in, the host-parasite relation of wilt diseases of plants. Especially from the fact *Fusarium oxysporum* f. *vasinfectum* produces fusaric acid ($C_{10}H_{13}O_2N$), a wilt toxin containing nitrogen(1, 6), detailed investigations on the effects of nitrogen source and rate for the growth of the fungus are considered to be significant to some purpose. Thus the writer attempted to investigate the effects of different N source and rate on the mycelial growth and sporulation of *Fusarium oxysporum* f. *vasinfectum* which is known to be a causal organism of the wilt disease of sesame and cotton.

Materials and Methods

Four N source were used in this study. The forms and reagent-grade salts were as follows: urea N, $CO(NH_2)_2$; ammonium N, $(NH_4)_2SO_4$; nitrate N, KNO_3; ammonium and nitrate N, NH_4NO_3. And then 9 different rates, 3, 6, 12, 25, 50, 100, 200, 400 and 800 meq of each N source were prepared to determine the effect of N rates. These nitrogen sources different in concentration were added in the N-free Preffer solution* with 3 % agar to solidfy the nutrient media of the fungus. The media prepared in this manner were sterilized in an autoclave at 15 lbs pressure for 30 minutes and separately placed in each petri plate in the amount of 10 ml. In this study each medium was used as it is without adjustment of pH because all of N source added in the basal nutrient solution were neutral salts. Exactly, all of the media indicated pH value of 5 ± 0.1 without remarkable differences each other.

The fungus used in this study was a virulent strain (No. 511) of *Fusarium oxysporum* f. *vasifectum* isolated from the root of wilted sesame in the college farm on Aug., 1961. On each medium, small amounts of the fragment of growing mycelium from 10 days old culture of the fungus on potato dextrose agar was transferred and incubated at 28+2°C. After 5 days culture, radial growth of the fungus were measured and also additional observations on the differences in the shape of colony and mycelial cell of the fungus were done. These experiments were replicated three times.

Results

1. Radial growth of aerial mycelium of the fungus on the media different in N source and rate

The results indicated that different N source and rate markedly affected the mycelial growth of the fungus. Nitrate N and urea N were appropriate N source for the mycelial growth of the fungus. Above all, nitrate N was considered to be the best N sourse because it is utilized in more extensive range of concentration in comparison with urea N by the fungus (Fig 1).

In other word, the fungus grew well on the media containing nitrate N in higher concentration than 400meq while it caused appreciacle reduction of mycelial growth on the media containing urea N in same level. On the other hand, the results indicated that ammonia N was of little avail for the fungus because it caused marked reduction of the mycelial growth irrespective of concentration. On the media containing N in the combination form of ammonium and nitrate, the fungus showed giowth rate corresponding to mean value of that on the media separately added nitrate N and ammonia N in same level. Anyhow, judging from the results, it is obvious that ammonia N causes marked reduction of the mycelial growth of *Fusarium oxysporum* f. *vasinfectum*. The results were summarized in Table 1.

Table 1. Average radial growth of the aerial mycelium of Fusarium oxysporum f. vasinfectum on the media different in N source and rate: 5 days cul ture at 28+2° C(menans of 3 replications)

N source / N concentration	$CO(NH_2)_2$	$(NH_4)_2SO_4$	KNO_3	NH_4NO_3
3meq.	2.5mm	0 mm	mm 4.0	3.5mm
6	19.0	7.0	19.2	20.5
12	27.8	15.7	28.7	23.5
25	33.8	13.5	33.2	28.5
50	30.5	9.6	33.7	23.3
100	28.7	8.5	31.0	19.5
200	27.6	8.3	29.5	17.0
400	22.0	10.7	29.7	18.8
800	14.2	12.3	25.6	15.8

2. Morphological differences of the colonies of the fungus on the media different in N source and rate.

The results indicated that different N source and rate also markedly affected the shape of colonis of the fungus. On the media containing urea N or nitrate N, the colonies of the fungus were circular in general. On the other hand the colonies of the fungus on the media containing ammonia N in more extensive range of concentr-

ation or ammonium and nitrate N in higher concentration than 50meq. lost in general their circular outline as radial growth continues and their margin became wavy and irregular. Especially on the media containing ammonia N alone in higher concetration than 25meq, the colonies of the fungus were unusually irregular and abnormal ones with margin deeply waved (Fig. 2). The results were summarized in Table 2. It was judged from the formation of unusual colony that ammonia N is of little avail for the growth of the fungus.

Tablle 2. Morphological differences of the colonies of Fusarium oxysporum f. vasinfectum on the media dnifferent in N source and rate: 5 days culture at 28+2°C

N source / N concentration	CO $(NH_2)_2$	$(NH_4)_2$ SO_4	KNO_3	NH_4NO_3
3meq	–	–	–	–
6	C	SW**	C	C
12	C	SW	C	C
25	C	DW***	C	C
50	C	DW	C	SW
100	C	DW	C	SW
200	C	DW	C	SW
400	C	DW	C	SW
800	C	DW	C	SW

* C: circular conloy, **SW: irregular colony with margin slightly weved, ***DW: irregular colony with margin deeply waved.

3. Morphological differences of mycelial cells of the fungus on the media different in N source and rate

From microscopy of mycelial cells of the fungus, it was known that different N source and rate affected shape of mycelial cells of the fungus. Abnormal bead-like mycelial cells (Fg. 3) were formed on the media containing ammonia N in higher concentration than 25meq while no such a bead-like mycelial cell was not formed on the media containing urea N, nitrate N, or ammonium and nitrate N. In other word, it was obviously recognized that make the colonies with margin deeply waved rule to accompany with abnormal bead-like mycelial cell without exception.

In an additional test transferred the bead-like mycelial cells on the media containing urea N or nitrate N, it was observed that normal elongate cells grow and develop from the bead like cells.

4. Differences in the relative amounts of sporulation of the fungus on the media different in N source and rate

It is known that the fungus usually produce three kinds of spore, namely macroconidium, microconidium and chlamydospore, on the appropriate culture media. On the media different in N source and rate, considerable differences were observed in the prodution of macro-and micro-conidium, especially of macroconidium while there were not noticeable differences in the production of chlamydospore.

On the media containing urea N or nitrate N in extensive range of concentration, both macro- and micro-conidia were abundantly or moderately produced. On the other hand, marked reduction of macro-and micro-conidia, espscially of macroconidia occured on the madia containing ammonia N. On the media containing ammonia and nitrate N, macroconidium were sparsely or moderately produced wnile microconidia were abundantly produced as well as on the media containing urea N or nitrate N or nitrate N alone. And no one of the media containing different N source and rate abundantly produced chlamydospore.

The results also indicated that spore production of the fungus, especially macroconidia production was affected by concentration of inorgannic N. In a word, much or less reduction of conidial production occured in higher concentration than 400 meq., or lower concetration than 25meq. irrespective of N source. The results were summarized in Table 3.

Thus, from relative amounts of sporulation, ammonia N was not considered to be an appropriate N surce for the growth of Fusarium oxysporum f. vasinfectum.

Table 3. Relative amounts of sporulation of Fusarium oxysporum f. vasinfectum on the media different in N source and rate : days culture at 28-2°C

N source / spore form / N concentration	$CO(NH_2)_2$			$(NH_4)_2SO_4$			KNO_3			NH_4NO_3		
	Ma	Mi	Ch	Ma	Mi	Ch	Ma	Mi	Ch	Ma	Mi	Ch
3 meq	0	0	0	0	0	0	0	0	0	0	0	0
6	1	2	1	0	1	1	1	2	1	1	2	1
12	1	3	1	1	1	1	1	3	1	1	3	1
25	2	3	1	0	0	2	2	3	1	1	3	1
50	3	3	1	0	0	0	3	3	1	2	3	0
100	3	3	0	0	1	1	3	3	0	2	3	0
200	3	3	0	0	1	0	3	3	0	2	3	0
400	2	3	0	0	0	1	2	3	0	1	3	0
800	2	3	0	0	0	1	1	2	0	1	1	0

a) relative amounts of sporulation O: no sporulation, 1: sparse, 2: moderate, 3: abundant.
b) spore-form Ma : macroconidium, Mi: Microconidium, Ch: chlamydospore.

Discussion

In an attempt to investigate the effects of different N source and rate for the growth of *Fusarium oxysporum* f. *vasinfectum*, the results indicated that different N source and rate markedly affected the growth of the fungus. Judging from some of growth response including radial growth of mycelium, shape of colony, shape of mycelial cell and sporulation, urea N and Nitrate N were better N sources than ammonia N or ammonium and intrate N for the growth of fungus. Among the N sources used in this study, ammonia N attracted the writer's attetion because it caused the formation of colonies with wavy margin and unusual bead-like mycelial cell in addition to marked reduction of radial growth of mycelium of the fungus. It was previously reported by some investigators(4, 7, 10, 11) that ammonia N is not an approprate N source for the growth of a few species of *Fusarium*. Hence such a dislike to ammonia N may be a nutritional characteristic which is common to *Fusaria*, and it also makes a striking contrast with a nutritional characteristic of *Verticillium albo-atrum*(3), another wilt fungus, which dislike urea N.

From the view of Hawker(2) and Naito et al(5), the abnormal colony with wavy margin was considered to be a characteristic of the "staling-type" growth of the fungus, resulting from the production of certain staling substance in general. In adiditon to the abnormal colony, the beadlike mycelial cell was also regarded as a characteristic of the staling-type growth from the results. Such an abnormal colony or bead-like mycelial cell on the media containing 2, 4-D and related phenoxy compounds was previously reported by some investigators (5, 8). Judging from the results of the present study, the effect of ammonia N to *Fusarium oxysporum* f. *vasinfectum* was similar to that of phenoxy compounds for the growth of some other fungi. Anyhow, it is of interest that ammonia N induce the staling type growth of the fungus irrespective of concentration.

Sanwal(10) and Luz(4) previously reported that *Fusarium oxysporum* f. lycopersici utilizes mainly the nitrate N in the first period of growth lasting some 2-3 weeks and later it also utilized the ammonia N. Judging from the results of this study, even if a culture medium contains nitrate N or urea N besides ammonia N, it is not an appropriate medium because the effect of ammonia N inducing unusual colony appears from the early stage of growth of the fungus. When N in the combination form of ammonium and nitrate was added in the nutrient solution for the growth of *Fusarium oxysporum* f. *vasinfectum* the utilization of ammonia N is not worth consideratior

while the abnormal colony originating from ammonia N is the first consideration.

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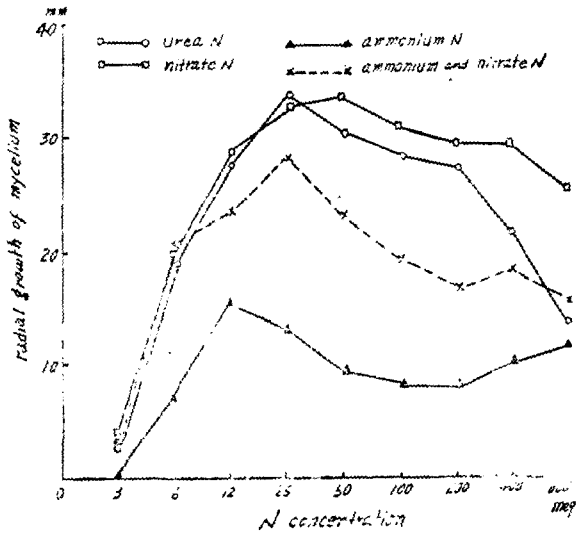
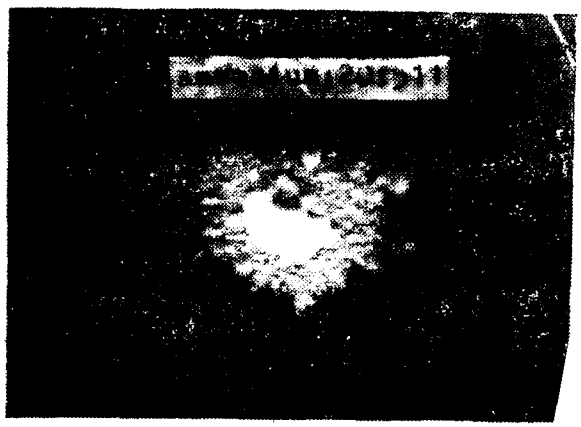


(Fig. 1)

(Fig. 2) an Abnormal colong of Fusarium oxysporum f. vasinfectum on the media Containing ammonia N



(Fig. 3) Bead-like mycelial cells in the Colong of Fusarium oxgsporum f. nasinfectum on the media Containing ammonia N

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要　約

窒素源의 種類 및 濃度가 참깨 萎凋病菌(*Fusarium oxysporum f. vasinfectum*)의 生育에 미치는 影響

朴 鍾 聲

우리나라에서 참깨나 목화에 寄生하여 萎凋病을 일으키는 *Fusarium oxsporum* f. *vasinfectum*의 生育에 미치는 窒素源의 種類및 濃度의 影響을 究明하고자 本硏究에 着手하였다.

本硏究의 結果로부터 窒素源의 種類 및 濃度의 差異가 *Fusarium oxysporum* f. *vasinfectum*의 生育에 큰 影響을 미친다는 것을 알았다. 供試한 4種의 窒素源 중에서 Nitrate N나 Urea N가 Ammonia N 또는 Ammonium and Nitrate N 보다 供試菌의 生育에 對하여 더욱 알맞는 窒素源이였다. 그 중에서도 Nitrate N는 他른 N源보다 훨씬 넓은 濃度範圍에서 本菌에 의하여 利用되므로 가장 알맞는 窒素源이라고 할 수 있다. 한편 Ammonia N는 供試菌의 菌糸生長이나 胞子形成의 顯著한 減少와 더부러 畸形的인 波形菌叢 또는 念珠狀菌糸細胞를 濃度에 關係없이 形成하므로 窒素源으로서의 利用價値가 거의 없다. Ammonia N가 供試菌의 生育에 미치는 影響은 波形菌叢 또는 念珠狀菌糸形成으로 보아 Phenoxy 化合物이 他른 眞菌의 生育에 미치는 影響과 恰似하다. Ammonium and Nitrate N도 50meq. 以上의 濃度에서 供試菌의 菌糸生長 또는 胞子形成을 減少시키는 한편 波形菌叢과 같은 異常生育이 多少間陰蔽되기는 하나 亦是 Ammonia N에서 由來하는 波形菌叢을 誘起하므로 알맞는 窒素源이라고 生覺할 수 없다. 따라서 Ammonia N 單獨 또는 他窒素源과 Ammonia N의 結合態는 *Fusarium oxysporum f. vasinfectum*의 生育을 위한 窒素源으로서는 不適當하다.

—抄　　錄—

麥類赤黴病에 對한 種子消毒試驗

鄭 鳳朝　　金 淵福　　李 淳炳

(要　旨)

赤黴病菌이 種子內部에까지 存在하여있지 않드라도 種子表面에는 無數한胞子나 菌糸等이 附着하여 第一次 發生을 助長하게 됨으로 種子消毒의 意義는 큰것이다. 그러므로 種子消毒方法으로 冷水溫湯浸, 沐浴湯, 藥劑에 依한 消毒으로 나누어 試驗하여 보았다.

1) 冷水溫湯浸法에 依한 種子消毒試驗 大麥에 있어서는 51°C에 5分間浸漬消毒함으로서 完全殺菌되었으며 小麥과 稞麥은 表皮가 없는關係로 48°C에서도 5分間으로 完全殺菌効果가 있었다. 發芽障害에 있어서는 53°C에 5分間까지는 支障이 없었으나 55°C에서는 顯著한 害가 있었다.

2) 沐浴湯浸에 依한 種子消毒試驗 처음 處理溫度 45°C에 10時間 浸漬消毒함으로서 麥種別(大麥, 小麥, 稞麥等)및 被害程度의 區別없이 完全殺菌되었다.

3) 藥劑에 依한 消毒試驗 벨크론外 數種의 藥劑를 供試하여 濃度와 處理時間을 달리하여 殺菌効果와 發芽障害에 關해서 調査해 보았을때 벨크론의 効果가 優秀하였다. 그러나 收穫後인 6月下旬에는 1000倍液에 6時間에서 完全殺菌되는것이 8月달에가서는 1000倍液에 1時間으로서도 前과 同一한 効果가 있었다. 이는 여러가지 環境要因에 依해서 病原性의 弱化를 意味하지 않는가 생각된다. 其他 昇汞 호루마링 硫酸銅等은 別로 實用効果가 없는듯 하였다. 發芽障害에 關해서는 濃度가 높을수록 또 處理時間이 길수록 發芽率이 低下되는 傾向이였다.

大麥에서는 벨크론 1000倍液에서 6時間 浸漬까지도 發芽率은 別로 低下되지않았으나 發芽勢는 時間이 길수록 나쁜傾向을 보여주었다. 稞麥에서는 3時間까지로 支障이 없었으나 6時間에서는 藥害가 있었으며 小麥에서도 稞麥과 같은 傾向이였다.

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