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FURTHER STUDIES ON ACREMONIUM WILT OF GRAIN SORGHUM

H.A. EL-SHAFEY, E.M. EL-ASSIUTY, A. ZEIN EL-ABEDEEN
THANAA F. IBRAHIM AND ZEINAB M.FAHMY

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

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Abstract

Acremonium-wilt of grain sorghum has become an important disease in Egypt and many of the sorghum-growing countries. The pathogen, *Acremonium strictum* Gams is a soil-borne pathogen invading the roots prior to colonizing the vascular tissues and is probably best controlled by host resistance.

Eight methods of inoculation were tested to identify a reliable, efficient and large-scale inoculation technique for the evaluation of genotypes in the field and in the greenhouse. Soil inoculation (infestation) was effective, uniform, and can be used for greenhouse evaluations, and probably for a small disease nursery. Stalk-inoculation using the toothpick technique was not effective in the greenhouse, but was effective, uniform and more practical for field evaluations. A soil drench with a spore suspension, 25 days after sowing, accompanied by root injury was also effective, but too laborious to be practical on a large scale. None of the other inoculation techniques tested was satisfactory.

Highly significant variation in virulence of 19 monospore isolates of *A.strictum*, from different hosts, was detected when the pathogenicity of these isolates was tested on four grain sorghum cultivars in the greenhouse, using the soil-infestation technique. Mean percentage infection ranged from 22.75% to 59.99%. Isolate No. 8 was the most aggressive, whereas isolates No. 11 was the least virulent. Dorado cultivar, on the other hand, was the most resistant (7.28% mean infection), whereas Giza-15, was the most susceptible (55.01%). The most aggressire isolate No. 8 (from grain sorghum), showing 90.0% infection on G-15, and only 5.3% infection on Dorado cultivar. Isolate No. 4 (from maize), exhibiting 55.01% infection on Giza-15, while it showed the highest infection on this resistant cultivar (32.46%). Isolate No. 3 (from maize) caused the highest infection (77.94%) on local-129, whereaas isolate No. 11 (from grain sorghum) showed the least infection (15.17%) on the same cultivar.

The reaction of 24 selected grain sorghum genotypes to *A.strictum* was tested in field trial, using toothpick stalk-inoculation. Seven entries namely; Dorado, BTX 623, BTX 631, ICSB-1, ICSB-14, ICSB-18 and ICSB-37 were highly resistant, while Giza-54 and Giza-114, were highly susceptible to infechin.

INTRODUCTION

Acremonium wilt, one of the most important diseases of grain sorghum (*Sorghum bicolor*). The disease was first described in Egypt by El-Shafey *et al.*

(1979) as a new vascular wilt caused by *Cephaloporium acremonium* Corda. Gams (1971) and this species was reduced to synonymy with *Acremonium strictum* Gams. The disease was described earlier by El-Shafey and Refaat (1978) as a stalk-rot caused by *C.acremonium*. Later, Frederiksen *et al.* (1980) and Natural *et al.* (1982) reported Acremonium wilt in the USA. Subsequently, the disease was reported in Argentina (Forbes and Crespo, 1982), in Mexico and the Sudan (Frederiksen, 1984).

Although *A.strictum* acts as a true vascular pathogen and could be easily isolated from the leaf veins, stalk-rotting fungi, however, often develop in wilted plants causing stalk-rot complex symptoms. In this respect, *A.strictum* acts as a predisposing agent. *A. Strictum* did not infect Sudan grass or broom corn, but could produce black-bundle condition in maize. The fungal hyphae were not detected in the maize stalk vessels.

Drying up of the leaf sheaths, reddish colour along the leaf veins, longitudinal reddish streaks on the stalks and gradual drying up of the plants are the main symptoms of Acremonium wilt (Fig. 1). Root-injury may facilitate and increase disease infection (El-Assiuty, 1982). Acremonium wilt was sporadically observed throughout the grain sorghum-growing Governorates, especially in Souhag and Al-Fayoum. Disease incidence may reach 50% or more in susceptible cultivars. Prematurely wilted plants do not usually form heads. Most of the tall Egyptian cultivars were susceptible, while most of the exotic short genotypes were moderately resistant or tolerant to the disease.

This research was initiated to test and identify effective, reliable and large scale inoculation techniques to be used in evaluating sorghum breeding genotypes for resistance to *A. strictum* in field and in greenhouse trials to demonstrate the pathogenic variation in 19 isolates of *A.strictum* and to test the reaction of some promising sorghum genotypes to Acremonium wilt disease.

MATERIALS AND METHODS

Methods of inoculation:

Eight methods were used to inoculate *A.strictum* into grain sorghum plants to identify an effective and practical method for the evaluation of breeding materials. Tests were conducted in greenhouse using two grain sorghum cultivars, (Giza -15 and Local-129). Five replicates, 20-cm-diam. pots, 5 plants each, were used. Concentration of the spore suspension was 2x10^4 cc. Drenching the soil was made with

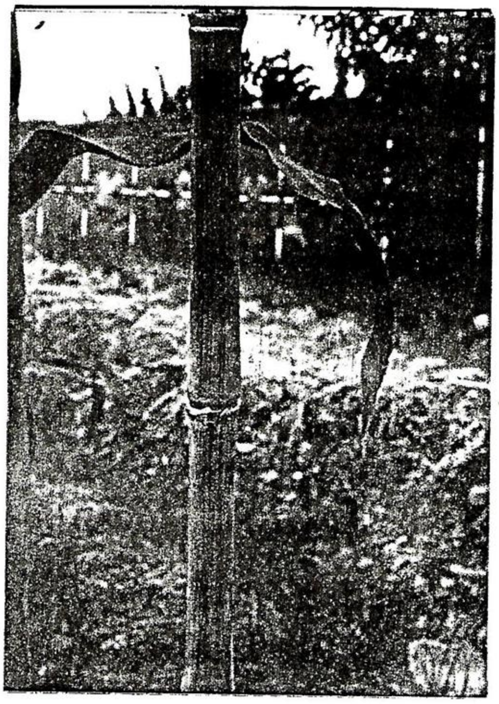

Fig. 1. Natural infection with Acremonium wilt of grain sorghum caused by *A.strictum*. The longitudinal reddish streak usually appear on one side of the stalk.

250 cc of the spore suspension/pot. Stalk-inoculation with toothpicks was tested in the greenhouse and in Giza field.

For soil-inoculation (infestation), inoculum of *A.strictum*, grown on moistened autoclaved sorghum grains, was mixed with the soil (fresh Nile silt) at the rate of 50 gm/pot. Stalk-inoculation was made by the toothpick technique described by Young (1943). Seed coating was tested by the alginate-pyrax seed-coating method adopted by Fravel *et al.* (1985). Disease rating were scored 90 days after sowing.

Pathogenic variation of *A.strictum* isolates:

Nineteen isolates of *A.strictum* were obtained from infected sorghum and maize plants. *A. strictum* could be easily isolated from the third or fourth internode above soil level. After surface disinfection with a piece of cotton moistened with alcohol, the infected internodes were cut lengthwise and the internal tissue was aspetically transferred onto PDA plates. The fungus could be more easily isolated from diseased sorghum leaves due to its vigorous growth in the vascular tissue of stalks, leaf sheaths, and leaves of the sorghum plants. Leaf pieces were washed in tap water, surface disinfected in 5% sodium hypochlorite for five minutes rinsed in sterile distilled water, and placed in PDA plates. Incubation was made at 28-29^{o}C. Pure, monospore cultures were obtained by streaking a diluted conidial suspension on plated PDA. Growth from isolated fungal colonies was transferred to fresh PDA. The pathogenicity of *A.strictum* monospore isolates was tested in the greenhouse using the soil-infestation method. Uninested pots served as a control. Disease ratings were recorded 90 days after sowing and data were transformed into arc sine.

Reaction of sorghum genotypes to *A.strictum*:

A number of commercial and promising sorghum genotypes were evaluated for resistance to *A.strictum* in Giza field. They were sown in two replicate rows, at the rate of twenty plants per row. Inoculation was made, 55-60 days after sowing, using the toothpick technique. After 90 days of sowing, the stalks were split long wise and disease ratings were recorded according to the 0.1-5.0 rating scale (0.1 = minimal infection, 5.0 = death of plant due to infection). Plants inoculated with sterile toothpicks served as a control.

Seeds of grain sorghum genotypes were obtained through the courtesy of the Sorghum Res. Section, ARC, Giza.

RESULTS AND DISCUSSION

Methods of inoculation :

Eight methods of inoculation were tested mainly in the greenhouse to develop efficient and practical techniques for screening grain sorghum breeding materials (genotypes). The eight methods differed greatly in their efficiency and practicality. They could be generally classified into effective and non-effective methods as follows:

a. Effective methods:

1. Soil-inoculation (infestation):

This method was effective and uniform. Wilt infection appeared 30-35 days after sowing (Fig. 2), 63.7% of Giza-15 and 20.0% of Local-129 plants were infected. Soil-infestation technique is well known and is used by many investigators, especially with soil-borne pathogens and was used by El-Shafey *et al*, (1978 & 1979) and El-Assiuty (1982) to test the pathogenicity of *A.strictum*, and to evaluate grain sorghum lines for resistance to Acremonium wilt. It can be used, on a large scale, for greenhouse evaluations, and probably for a small disease nursery plots.

Soil infestation, was also utilized, on a large scale, in the National Maize Program to breed for late wilt resistance. (Abd-El-Rahim, 1971 and El-Shafey *et al.*, 1988).

2. Stalk-inoculation (using toothpicks):

This method did not work in the greenhouse because the thin stalks of the potted plants which usually break after the toothpick is inserted into the basal internode. Natural *et al.* (1982) used different techniques of stalk-inoculation to test the pathogenicity of *A.strictum* isolates. They showed that sorghum plants inoculated with a hypodermic injection of conidial suspension above the first node of the stalk resulted in a rapid wilting, whereas introducing conidial suspension between the leaf sheath and the stalk resulted in minor disease symptoms. The stalk-inoculation with toothpicks, however, was very efficient, uniform and practical with field-grown plants having thick stalks (Fig. 3). It was used, on a large scale, to screen genotypes of the grain sorghum breeding program for resistance to stalk-rots caused by *C.acremonium* (*A.strictum*), *Fusarium moniliforme*, and *Sclerotium bataticola*.

The toothpick stalk-inoculation procedure was followed by most investigators to screen for resistance to charcoal rot of sorghum caused by *Macrophomina phase-*

Fig. 2. Artificial infection with Acremonium wilt of grain sorghum potted plants using the soil-inoculation technique control pot on the left.

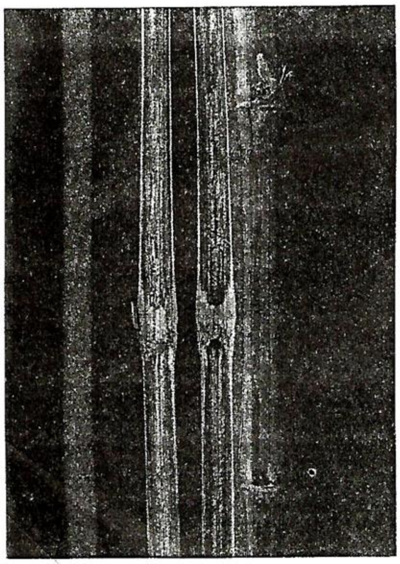

Fig. 3. Two field-grown plants of grain sorghum inoculated with A.strictum using the toothpick stalk-inoculation technique. The plant on the right was cut lengthwise to show internal symptoms.

olina. Edmunds *et al.* (1964), however, had some reservations about using this technique with charcoal rot. He claimed that toothpick inoculation and other methods where inoculum is introduced into the plant through the stalk are unsatisfactory, primarily because they do not closely simulate the natural infection process, which begins in the roots and only later goes up to the stalk. Furthermore, the level of disease development with toothpick inoculation is usually less than that which occurs naturally and is, therefore, unsatisfactory for assessing resistance that could be useful under natural disease incidence.

3. Soil drench with root injury:

Drenching the soil with spore suspension, after injuring the roots by scraping the soil around the plants with a blunt scalpel, 25-days after sowing, was effective; 38.0% of Giza-15 and 35.0% of Local-129 plants were infected. Natural *et al.* (1982) showed that drenching around injured roots with spore suspension resulted in rapid infection. El-Assiuty (1982), on the other hand, stated that highest infection with Acremorium wilt was obtained when the inoculum was applied into the soil 30-days after sowing, whether the roots were injured or not. At later stages of plant growth (45-days after sowing), however, root injury facilitated and increased infection. This method, however, can be used, on a narrow scale, in greenhouse tests.

b. Non-effective methods:

1. Seed-infestation:

Seeds were soaked in spore-suspension for 18-20 hours before sowing. This technique was not effective. Both cultivars showed no infection.

2. Soil drench at sowing:

The spore suspension was poured onto the soil at sowing. This method was not effective resulting in 0.0% infection of Giza-15 and 7.0% infection of Local-129 plants. Frederiksen (1984) mentioned that blending conidia with steamed soil at sowing, failed to cause disease, but disease did develop when seedlings were transplanted into infested soil, indicating that root wounding may be necessary for infection (H.J. Kim and J.K. Mitchell (Dept. of Plant Pathology and Microbiology, texas A & M Univ., Personal communication).

3. Soil drench root-injury:

Spore-suspension was poured around the plants, 25 days after sowing. This method of inoculation was slightly effective. Only 10.0% of Giza-15 and 3.0% of local-129 plants were infected.

4. Foliar-spraying:

Foliar spraying with spore-suspension, 30-days after sowing, after clipping the leaves with scissors was not effective; infection appeared as reddish streaks along the veins of the leaf blades, and in some cases progressed downwards to the leaf sheaths, but at 90 days after sowing no signs of infection were observed in the stalks. Natural *et al.* (1982) showed that infection developed very slowly following inoculation by foliar spraying, whereas inoculation at the base of the plants (above the first node) increased their susceptibility.

5. Seed-coating:

This method adopted by Fravel *et al.* (1985) was not effective. Less than 5.0% of the plants of both cultivars were infected.

A.strictum was recovered from discolored diseased leaves of inoculated plants.

Pathogenic variation of *A.strictum* isolates:

The pathogenic variation in 19 monosporic isolates of *A.strictum* was studied. The isolates, obtained from different Governorates of the Delta and Upper Egypt, were isolated from diseased grain sorghum, sweet sorghum and maize plants. The pathogenicity was tested on 4 grain sorghum cultivars. The selection of the these cultivars was based on the records of the Maize Disease Res. Section which indicated different degrees of susceptibility.

The results presented in Table (1) showed that wide variation in the pathogenicity of *A.strictum* isolates does exist. The mean infection percentage of the different isolates, on the tested grain sorghum cultivars, ranged from 22.75% to 59.99%. Isolate No. 8 was the most aggressive, whereas isolate No. 11 was the least virulent with highly significant differences. Dorado cultivar, on the other hand, showed the highest degree of resistance (Mean infection 7.28%). It exhibited 0.0% infection against seven (out of 19) tested isolates, whereas Giza-15 was the most susceptible to *A.strictum* isolates (55.01%).

The most aggressive isolate No. 8 (from grain sorghum) which caused the highest infection percentage on Giza-15 (90.0%), almost failed to infect the resistant Dorado cultivar (5.33%). Isolate No. 4 (from maize), exhibiting much less infection on Giza-15 (55.01%), showed the highest infection percentage on the same Dorado cultivar (32.46%). Isolate No. 3 (from maize) caused the highest infection percentage (77.94%) on Local 129 cultivar, whereas isolate No. 11 (from grain sorghum) showed the least infection (15.17%) on the same cultivar. These big differences in virulence between *A.strictum* isolates, from different hosts, on the grain sorghum cultivars indicate that different pathotypes of this pathogen do exist. *A.strictum* isolates were recovered from discolored diseased leaves of the inoculated plants and were found typical of the original cultures.

Table 1. Pathogenic variation in 19 isolates of *A.strictum* on four grain sorghum cultivars, under gressnhouse conditions.

No.	Location	Host	% Infection (arc sine)/ cultivar				Mean
			Giza-15	Dorado	Line-113	Local-129	
1	Dakahlia	Maize (corn)	49.04	0.00	48.51	60.21	39.44
2	Beni-Suer	Maize	39.44	0.00	28.23	33.48	25.29
3	Beni-Suer	Maize	67.37	12.06	75.18	77.94	58.14
4	Beheira	Maize	55.01	32.46	51.34	45.36	46.04
5	Menia	Maize	74.36	20.63	67.33	63.20	56.38
6	Menia	Grain sorghum	61.00	5.33	59.06	49.82	43.80
7	Menia	Sweet sorghum	45.00	10.83	49.74	45.90	37.87
8	Fayoum	Grain sorghum	90.00	5.33	71.91	72.72	59.99
9	Giza	Grain sorghum	55.47	10.66	42.72	50.86	39.93
10	Giza	Grain sorghum	38..51	5.33	38.06	25.03	26.73
11	Giza	Grain sorghum	42.53	0.00	33.31	15.17	22.75
12	Giza	Grain sorghum	47.48	10.27	40.96	39.98	34.67
13	Souhag	Grain sorghum	68.70	9.91	47.96	46.91	43.37
14	Giza	Grain sorghum	53.12	0.00	49.61	32.02	33.69
15	Giza	Grain sorghum	46.91	10.27	44.00	33.69	33.72
16	Giza	Grain sorghum	58.49	0.00	40.11	32.35	32.74
17	Qalubia	Maize	48.98	5.33	41.97	29.03	31.33
18	Qalubia	Maize	51.76	0.00	36.88	27.30	28.99
19	Assiut	Grain sorghum	51.94	0.00	45.91	25.74	30.90
	Mean		55.01	7.28	48.04	42.60	

L.S.D. at		5%	1%
for	Cultivar (C)	4.33	5.71
	Isolates (I)	9.43	12.44
	C x I	18.87	NS

In a cross-inoculation experiment, El-Assiuty (1982) found that *A.strictum* isolates, whether from grain sorghum or from maize, were more virulent to grain sorghum than to maize cultivars. All isolates produced typical vascular wilt sysmptoms in sorghum, but only minor black-bundle symptoms in maize. The isolates from grain sorghum, however, showed a slight increase in virulence to sorghum plants.

Mansour *et al.* (1986) stated that significant differences in pathogenicity between 17 monosporic isolates of *A.strictum* (*C.acremonium*) appeared when these isolates were tested against three of grain sorghum cultivars.

The aggressive monospore isolates of *A.strictum* will be utilized for the evaluation of grain sorghum breeding genotypes to identify sources of resistance to Acremonium wilt. In disease control programs using host resistance, the study of the pathogen variation is a very important factor and, therefore, the present study should be continued to collect and study more isolates of *A.strictum* in order to identify different pathotypes (possible races) of this pathogen, that can overcome specific sorghum genes of resistance.

Evaluation of grain sorghum genotypes for resistance to *A.strictum*:

The reaction of 24 selected grain sorghum genotypes to A.strictum was tested in Giza field using the toothpick stalk-inoculation technique. Infection was verified by isolating the pathogen from a number of diseased plants taken at random.

Fig. 2. Evaluation of selected sorghum promising genotypes for Acremoniuum wilt resistance, under field conditions.

No.	Pedigree	Disease rating*	No.	Pedigree	Disease rating*
1	BTX 623	0.5	13	EGH-2	0.8
2	BTX 629	0.8	14	EGH-2	1.0
3	BTX 631	0.5	15	G.POP.III-2/78	2.5
4	BTX 635	0.8	16	Fam 334	2.0
5	ICSB-1	0.5	17	Sel. 113	1.0
6	ICSB-14	0.5	18	Local 129	1.0
7	ICSB-18	0.5	19	Local 245	2.0
8	ICSB-20	1.0	20	Giza-15	2.0
9	ICSB-37	0.5	21	Giza-54	4.0
10	ICSB-112	0.8	22	Giza-114	4.0
11	Dorado	0.2	23	Giza-123	2.0
12	EGH-1	0.8	24	NES 1007	0.8

* Numerical grades expressing degrees of infection from 0.1 (minimal infection, indistingusishable from that of a sterile toothpick) to 5.0 (death of plant due to infection). Grades 1.0 or less are considered resistant.

The results as illustrated in Table 2 showed that 17 entries were resistant (grad 1.0 or less), of these 7 entries namely; Dorado, BTX 623 BTX 631, ICSB-1, ICSB-14, ICSB-18, and ICSB-37 were highly resistant (grade 0.5 or less). Giza-54 and Giza-114, on the other hand, were highly susceptible to the disease showing almost death of plants. The remaining entries exhibited comparable degrees of susceptibility. El-Assiuty (1982), using the toothpick-inoculation, showed that sorghum cultivars NES 1818 and NES 1324, were highly resistant to *A.strictum*, whereas L 102, L129 and Hybrid-113 were highly susceptible.

In previous evaluation (unpublished data), L129 and Giza-15 showed high degree of susceptibility (grade 4.0). It is obvious that relative resistance exhibited by these entries, in the present test (grade 1 and 2, respectively), could be due to the genetic improvement that has been achieved through the sorghum breeding program.

More sorghum genotypes will be tested for reaction to *A.strictum*, by soil and stalk inoculation, to identify a wide variety or resistant sources in order to enhance sorghum breeding program for disease resistance.

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دراسات أخري علي مرض ذبول الأكريمونيم في الذرة الرفيعة

هارون عبد المنعم الشافعي، الهامي مصطفي الأسيوطي،
علي زين العابدين، ثناء فهمي إبراهيم، زينب محمد فهمي

معهد بحوث أمراض النباتات – مركز البحوث الزرعية – الجيزة – مصر.

يعتبر ذبول الأكريمونيم من أهم أمراض الذرة الرفيعة في مصر وبعض دول العالم الأخري. وقد تم تعريف الفطر المسبب لهذا المرض (أكريمونيم استريكتم) لأول مرة بمعرفة الشافعي وآخرين سنة ١٩٧٩ كأحد فطريات الذبول الوعائي الكامنة في التربة حيث يهاجم الجذور ثم ينمو بقوة ويحتل أوعية الخشب في الساق والأوراق. ولايصيب هذا الفطر الذرة الشامية (كذبول وعائي) ولكنه إحيانا ما يسبب أعراض اسوداد الحزم الوعائية فيها دون تأثير اقتصادي يذكر علي المحصول.

وتظهر الأعراض الأولي لهذا المرض علي الذرة الرفيعة بعد حوالي ٤٠ – ٥٠ يوماً من الزراعة في شكل جفاف أعماد الأوراق السفلي اولا وتلون عروق النصل باللون الأحمر الغامق، ثم يتلون الساق ويتجعد ويجف تدريجياً وتظهر عليه خطوط طولية بلون بني محمر، وقد لا تتكون الرؤوس (الكيزان) علي الإطلاق في الإصابات الشديدة (المبكرة). ويظهر المرض في كثير من مناطق زراعة الذرة الرفيعة في مصر وخاصة في محافظتي سوهاج والفيوم، وقد تصل نسبة الإصابة به إلي ٥٠٪ علي بعض الأصناف شديدة القابلية للإصابة.

وقد أجري هذا البحث لإختبار فعالية ثمانية من طرق الحقن الصناعي للفطر المسبب لتحديد طريقة (أو طرق) مناسبة لإستخدامها في تقييم درجة مقاومة سلالات وأصناف الذرة الرفيعة بغرض تعريف مصادر وراثية مقاومة لهذا المرض، وكذلك لدراسة التباين في القدرة المرضة لعدد ١٩ من عزلات الفطر المسبب وأيضا لإختبار درجة مقاومة ٢٤ من سلالات وأصناف الذرة الرفيعة للمرض.

وقد أظهرت النتائج أن حقن (تلويث) التربة بلقاح الفطر طريقة فعالة، متجانسة، وعملية لتقييم سلالات وأصناف وخجن الذرة الرفيعة بالصوبة الزجاجية، وأيضاً في مساحة محدودة بالحقل، أما طريقة حقن الساق، فوق سطح التربة مباشرة بسلاكات الأسنان الحاملة لنمو الفطر المسبب فقد أظهرت فعالية كبيرة كطريقة عملية يمكن إستخدامها لإختبار درجة مقاومة أصناف الذرة الرفيعة علي نطاق واسع في حقول التربية لإستنباط الأصناف المقاومة. وقد كانت طريقة إضافة معلق جراثيم الفطر المسبب إلي التربة مع تجريح الجذور طريقة فعالة أيضا في احداث الإصابة غير أنها لم تكن عملية تماماً واكتنفتها بعض الصعوبات في التطبيق، ويمكن إستخدامها في اي حال علي نطاق ضيق بالصوبة الزجاجية. أما باقي طرق الحقن الأخري فقد كانت إما ذات فعالية طفيفة أو عديمة الفعالية علي الإطلاق في إحداث الإصابة.

وقد أظهرت نتائج إختبار التباين في القدرة الممرضة لعدد ١٩ من العزلات النقية لفطر ذبول الأكريمونيم (معزولة من مناطق وعوائل مختلفة) علي عدد ٤ من أصناف الذرة الرفيعة تبايناً واسعاً (معنويا) في قدرة هذه العزلات علي إحداث الإصابة، فقد تراوح متوسط النسبة المئوية للإصابة في العزلات المختلفة بين ٢٢,٧٥٪ و ٥٩,٩٩٪، وقد كانت العزلة رقم ٨ أشد العزلات إحداثاً للإصابة (عدوانية) بينما كانت العزلة رقم ١١ أقلها في شدة الإصابة. ومن ناحية أخري، فقد أظهر صنف الذرة الرفيعة دورادو أعلي درجات المقاومة لعزلات الفطر المختلفة بينما كان الصنف جيزة ١٥ أكثر الأصناف قابلية للإصابة. وقد أظهرت العزلة رقم ٨ (الأكثر عدوانية) والمعزولة من الذرة الرفيعة إصابة قليلة (٥,٣٣٪) علي الصنف المقاوم دورادو بيننما أظهرت العزلة رقم ٤ المعزولة من الذرة الشامية أعلي نسبة إصابة (٣٢,٤٦٪) علي نفس هذا الصنف المقاوم، كذلك أظهرت العزلة رقم ٣ (من الذرة الشامية) أعلي نسبة إصابة (٧٧,٩٤٪) علي الصنف محلي-١٢٩ بينما أظهرت العزلة رقم ١١ (من الذرة الرفيعة) أقل نسبة للإصابة (١٥,١٧٪) علي نفس هذا الصنف.

أما بالنسبة لإختبار درجة مقاومة ٢٤ من سلالات وأصناف وهجن الذرة الرفيعة لمرض ذبول الأكريمونيم بإستخدام حقن الساق بسلاكات الأسنان بحقل مزرعة الجيزة فقد أظهرت النتائج أن ١٧ من هذه الأصناف كانت مقاومة أو عالية المقاومة بينما أظهر الصنفان جيزة - ٥٤ ، جيزة - ١١٤ قابلية شديدة للإصابة بهذا المرض.