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CLEISTOTHECIA PREVALENCE OF POWDERY MILDEW CAUSED BY Erysiphe betae ON SUGAR BEET FIELDS IN EGYPT.

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ABSTRACT

The first appearance of powdery mildew symptoms on sugar beet plants usually occurred on late winter and early summer in Dakahlia governorate, Egypt. On the basis of the observation, field study that conducted between 1st week of April and May in 2002 and 2003 to assess the incidence and severity of the mildew on some commercial cultivars. Results showed a constant increase of mildew incidence and severity that occurred regularly as the time pass. There were a significant differences in mildew incidence and severity between plots in edges and inside the field. In late April, Cleistothecia were formed in abundance on all inspected cultivars in 2002 and 2003. Differences in cleistothecia density were observed on old and young leaves or in abaxial and adaxial surfaces of the leaves. Percentage of mature cleistothecia ranged from 35 to 55% in early inspection and reached 85% in late inspections. Percentages of viable cleistothecia were 70.8; 26.8 and 48.8% for the categories of fresh; soil stored and indoor stored cleistothecia; respectively. Viable cleistothecia from the three categories used in the pathogenicity test showed different results in percentage of reproduced powdery mildew symptoms on plants exposed to ascospores inoculum.

These results clearly show that cleistothecia of Erysiphe betae (Vanha) Weltzien can be considered as important source of inocula for powdery mildew of sugar beet in Egypt. Prevalence and perennation of cleistothecia could sustain the possibility of the development of new, and perhaps more virulent races of mildew.
Keywords: sugar beet, powdery mildew; cleistothecia, Erysiphe betae.

INTRODUCTION

Sugar beet (*Beta vulgaris* ssp. vulgaris L.) is a globally important crop producing 27% of world sucrose supplies. It is grown in Europe, North America, Chile, Uruguay, China, the Middle East, North Africa and countries of the former Soviet Union (Cooke and Scott, 1993). The potential geographical range of powdery mildew infection has been predicted using meteorological data (Drandarevski, 1969) and the fungus has steadily spread within these limits. Infection is especially damaging in areas with arid climates, for example in Mediterranean countries, the Middle East and in California (Ruppel, 1995), causing sugar yield losses of up to 30% (Weltzien and Ahrens, 1977). Its prevalence continued to increase, possibly because of several factors including increasing crop susceptibility, changing climatic conditions or cultural practices favouring disease development, or a change in pathogen virulence (Byford, 1996, Francis, 2002). Cleistothecium production is common in some countries, (Mamluk and Weltzien, 1973). In other areas, mildew may have been present for several years before cleistothecia were discovered, for example in England (Byford and Bentley, 1976), and in the USA, where they were found initially in only one field (Coyler *et al.*, 1975), and not seen again for many years (Ruppel, 1995), then became more widespread (Gallian & Hanson, 2003 and Harveson, 2004).

Sugar beet powdery mildew was first recorded in Egypt by El-Kazaz *et al.*, 1977. The disease became widespread and causes considerable losses over the last few years especially in fields cultivated in late sowing dates. Like most powdery mildew fungi, Erysiphe betae (Vanha) Weltzien has relatively narrow host range. Other forms of Beta vulgaris, such as wild beet (Beta vulgaris subsp. vulgaris var. vulgaris) that grow as a weed in Egypt; fodder beet (B. vulgaris subsp. vulgaris var. alba) and table beet (B. vulgaris subsp. vulgaris var. conditiva). This work reports the occurrence and possible role of E. betae cleistothecia in epidemic of sugar beet powdery mildew in Egypt. To estimate the mildew incidence, severity and to quantity the density and viability of cleistothecia on some commercial sugar beet cultivars.

MATERIALS AND METHODS

Inspection of powdery mildew disease symptoms was done on late winter and early summer of 2002 and 2003 growing seasons. The cleistothecia of E. betae were observed on infected sugar beet cultivars; Oscar; Pleno and Betapoly cultivated at Mansoura University farm, Egypt. Also, disease symptoms with cleistothecia were showed on other commercial sugar beet farms and on wild beet grown as weed nearby the sugar beet field experiment.

Incidence and severity

Weekly records of disease incidence and severity were assessed from its onset usually around the mid of March until about 1st May. Diseases incidence was determined in 10 plots located at edges and inside the field. In each plot, disease survey were done in 10 rows. Disease incidence was expressed as percentage infection (Number of infected plants / total number of inspected plants per row X 100). Disease severity was determined following the visual diagram key of diseased leaf surface prepared by Hills *et al.*, (1980) in hundred leaves detached from 25 plants selected randomly from each plot.

Data were analyzed with the Statistical Analysis System (SAS Institute 1988). All multiple comparisons were first subjected to analysis of variance (ANOVA) and means were compared using Duncan's multiple range tests.

Inspection and assessment of cleistothecia

Upper and lower leaves of infected plants were inspected for cleistothecia that started to appear on late April. Twenty-five samples of infected young and old leaves from 10 plots located at edges and inside the field were inspected for cleistothecia using hand lens (10X). Presence of cleistothecia expressed as the mean number of cleistothecia per square area of leaf (1 cm2). The means was used to categorize density of cleistothecia that calculated and scaled to three levels; low (L = = 50 in early inspection or =100 in late inspection) moderate (M = = 180 or = 250) and high (H = > 300 or > 500) for early and late inspection, respectively.

Examination of cleistothecia maturity and viability

During early and late inspection, samples from young and old leaves with low to high densities of cleistothecia were used to assess maturity development of cleistothecia using a dissecting microscope (50X).

Percentages of mature and immature cleistothecia were calculated. Also, a compound microscope was used to check the viability of asci and ascospores in 100 mature cleistothecia removed from leaf pieces and crushed under a glass slide covers. The asci were categorized as asci containing viable mature spores had vaculated cytoplasm and no oil droplets; immature spores with granular cytoplasm or degenerate spores containing dark cytoplasm with numerous lipid droplets. The percentage of cleistothecia containing ascospores of each category was recorded.

Pathogenicity

Pathogenicity test was made to proof the infectivity of the ascospore from mature cleistothecia under greenhouse conditions in May, 2003 for freshly collected mature cleistothecia using disease-free sugar beet plants. Moistend leaf pieces with mature cleistothecia were suspended over healthy leaves of 3 months old plants (5 replicates). In April 2004, the same experiment were repeated using stored cleistothecia from the previous season in paper bag under room condition or stored as leaf debris in soil during summer. Before inoculation step, cleistothecia were tested for ascospore viability by compound microscope as indicated before. Development or reproducing of disease symptoms was monitored daily for 2 weeks and number of infected plants were recorded.

RESULTS

In Egypt, powdery mildew appears on late sowing dates in sugar beet fields that harvested in early summer. The first sign of mycelium of powdery mildew on leaves usually is not seen until 10-12 wk after emergence. Subsequently, disease increases rapidly especially in fields has no chemical control or good cultural practices.

Incidence and severity

Data presented in Table 1 summarized incidence and severity of mildew that assessed in April / May 2002 and 2003 in a comparison between plants at edges and inside the field.

In 2002 growing season; during the first week of April the symptoms was visible on plants at field edges more than inside the field. Percentage infection at field edges ranged from 2.1 to 4.5% at 1st week of April then reached gradually to 37 and 39.5% after one month on Oscar and Pleno cultivars, respectively. Inside the field; percentage infection was tiny and negligible at 1st and 2nd weeks of April then started to increase to reach 24% and 32.8% after one month on Oscar and Pleno cultivars, respectively.

In 2003 growing season; Betaboly was another available cultivar used to study powdery mildew. This cultivar was more susceptible to powdery mildew. Disease incidence was low (1.3% and 6.%) on 1st week of April then gradually increased to reach 30 and 41.5% at 1st week of May on plants at edges and inside the field, respectively.

Data presented in Table 1 showed the severity of powdery mildew rated 4 times during April / May 2002 and 2003. In 2002, there were no significant differences between Oscar and Pleno cultivars in ratings taken on 1st and 2nd weeks of April for plants inside the field; while it was highly significant different at field edges where Pleno cv was higher than Oscar especially in severity ratings had = 10%; = 25% and = 50% of symptoms on plants. In 2003, disease severity ratings on Betaboly cv showed higher values of disease severity on plants grown at field edges and inside the field compared with Oscar and Pleno cvs especially in 2nd week of April.

Table (1): Incidence and severity of powdery mildew on some sugar beet cultivars during 2002 and 2003 growing seasons at Dakahlia governorate, Egypt.

Inspection date		Cultivars					
		Oscar		Pleno		Betaboly	
		2002				2003	
		Disease incidence *					
		Field edges	Inside field	Field edges	Inside field	Field edges	Inside field
April, 1st week		2.1 d	0 3 c	4.5 d	0.2 d	6 3 d	1 3 d
April, 2nd week		5 7 c	1 3 c	18 2 c	3 6 c	23 3 c	11 3 c
April, 3rd week		25.8 b	18 b	25.7 b	20.8 b	33.5 b	24.5 b
May, 1st week		37 1 a	24 1 a	39 5 a	28.6 a	41 5 a	30 a
	Severity scale	Disease severity **					
April, 1st week	≥ 10%	0 9 f	0 3 f	3.8 ef	0 5 gh	1 5 hi	0 8 ef
	≥ 25%	1 1 f	0 f	2.2 g	0 h	2 3 gh	0 f
	≥ 50%	0.1 f	0 f	0.3 h	0 h	0 6 ji	0 f
	≥ 75%	0 f	0 f	0 h	0 h	0 j	0 f
	≥ 100%	0 f	0 f	0 h	0 h	0 j	0 f
April, 2nd week	≥ 10%	3 d	0 6 f	10 5 b	1.5 fgh	9 1 d	6 c
	≥ 25%	2.4 de	0 7 ef	5 1 e	2 1 f	9.2 d	4 d
	≥ 50%	0 3 f	0 f	2.7 fg	0 8 fgh	5 4 f	1 2 ef
	≥ 75%	0 f	0 f	0 h	0 h	0 j	0 f
	≥ 100%	0 f	0 f	0 h	0 h	0 j	0 f
April, 3 rd week	≥ 10%	7.7 b	12.3 d	3.8 ef	9 8 ab	0 j	8 6 b
	≥ 25%	5.9 c	5.4 c	8.4 cd	7 1 d	5 6 f	8 4 b
	≥ 50%	7 bc	0 3 f	7.4 d	3 9 e	14 3 b	5 8 c
	≥ 75%	2.9 d	0 f	4 ef	0 1 h	8 5 ed	1 5 ef
	≥ 100%	2 3 de	0 f	2.1 g	0 h	3.1 g	0 6 ef
May, 1st week	≥ 10%	0 f	5 8 c	0 h	4 7 e	0 j	4 7 cd
	≥ 25%	7 3 bc	13 3 a	4 1 ef	10 5 a	2 6 gh	15 4 a
	≥ 50%	12.1 a	3.2 d	9.6 bc	8.6 bc	18 5 a	7 8 b
	≥ 75%	11 3 a	1 5 e	12 9 a	7 3 cd	10 8 c	2.1 e
	≥ 100%	6 4 bc	0.3 f	13 a	1.7 fg	7 5 e	1.5 ef

* , ** Values of disease incidence and disease severity followed by the same letter(s) within each column are not significantly different *(P 0 05)* according to Duncan's multiple range test.

Disease severity rating values in 3rd and 4th week started to increase in plants at both field edges and inside the field. The severity ratings exceeded 50% and reached 100 % on infected plants of all cultivars in the 4th week (1st wk of May) in 2002 and 2003. In addition; there were highly significant differences between disease severity rating values taken for plants at edges and inside the field as shown in Table1. In general; the number of plants had values exceed 75% of disease severity scale were significantly higher in field edges than inside the field.

Inspection and assessment of cleistothecia:

Cleistothecial initials at low density were first observed in early April; and cleistothecia developed in moderate and high density on all inspected cultivars as time passed. Both severely affected plants and plants without much visible mycelium had abundant cleistothecia on the lower and upper leaf surfaces. As shown in Table 2; densities of cleistothecia on old leaves were higher than young leaves.

Table (2): Assessment of cleistothecia density on infected leaves of some sugar beet cultivars in different inspection times.

Cultivar	Inspec tion date	Leaf age	Total No. leaves	No. Leaf with cleisto-thecia	No. leaves in each category of cleistothecia density					
					Leaf upper surface			Leaf lower surface		
					L*	M	H	L	M	H
Oscar	(Early) April, 2[nd] wk	Old	110	10	0	0	0	10	0	0
		Young	85	0	0	0	0	0	0	0
	(Late) May, 1[st] wk	Old	120	78	9	12	2	5	20	30
		Young	80	21	3	5	0	3	2	8
Pleno	(Early) April, 2[nd] wk	Old	115	22	0	4	0	6	12	0
		Young	75	5	2	0	0	0	3	0
	(Late) May, 1[st] wk	Old	140	91	4	6	10	5	15	51
		Young	85	32	2	2	5	0	10	13
Betapoly	(Early) April, 2[nd] wk	Old	160	46	3	5	0	3	11	24
		Young	120	8	0	0	0	2	6	0
	(Late) May 1[st] wk	Old	185	100	0	16	7	8	36	33
		Young	145	43	0	8	2	3	22	8

* Twenty-five plants showed infection on young and old leaves were assessed for cleistothecia density or its mean number per leaf scaled to three levels ; low (L = <50or 100); moderate (M = < 180 or 250 and high (H = > 300or 500) for at early and late inspection , respectively .

Also, it was higher on lower surface more than upper surface of leaf. Among cultivars; cleistothecia density were abundant on Pleno cv. more than Oscar cv. in 2002 growing season. In 2003 Betaboly cv was the most susceptible cultivar had high density of cleistothecia compared with cultivars inspected in 2002 or other commercial cultivars observed in farmer fields in 2003.

Examination of cleistothecia maturity and viability:

Cleistothecia initials were yellow to light orange in color, then turned brown to black and developed irregular branched appendages (Fig1).

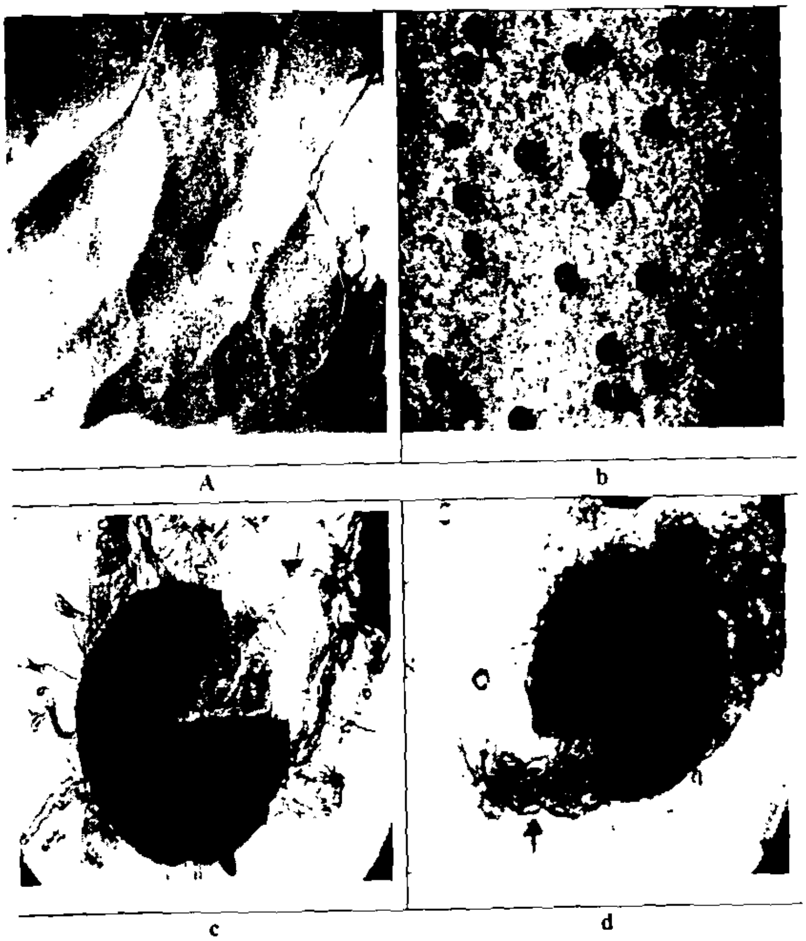

Fig. (1): Powdery mildew cleistothecia of Erysiphe betae on sugar beet leaves (a-b) and their Ascospores inside Asci (40X) (c-d).

Mature cleistothecia were usually dark brown to black with appendages, and contained asci with 2-4 ascospores per ascus. Both young and old leaves had mature and immature cleistothecia on the lower and upper leaf surfaces. As shown in Fig.2 percentage of mature cleistothecia ranged from 35 to 55% in early inspection and reached 85% in late inspection at 1st week of May in all infected leaves of all cultivars. Immature percentage were abundant (45 – 65%) in early inspection date (1st week of April) then decreased by time to 15 – 25% of total cleistothecia number.

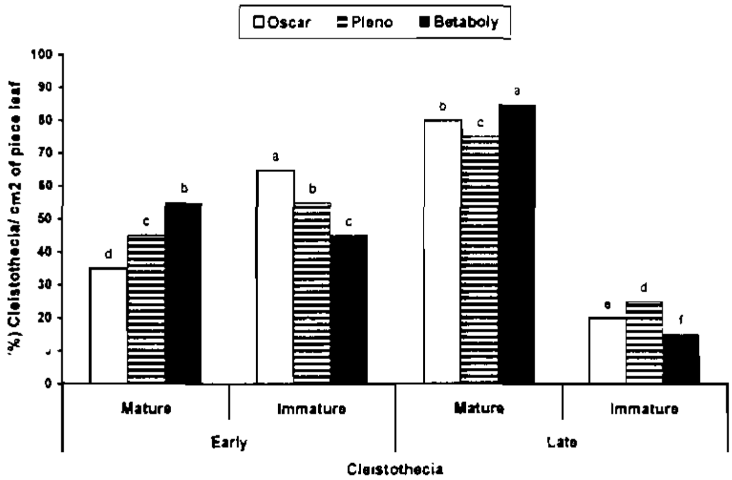

Fig. (2): Assessment of maturity in early and late inspection for cleistothecia on sugar beet cultivars; Oscar, Pleno in 2002 and Betapoly in 2003 growing seasons. Bars for each category of cleistothecia in both early and late inspection topped with the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

Evaluation of viability for fresh and stored cleistothecia categories using the compound microscope examination showed that percentage viable cleistothecia were 70.8; 26.8 and 48.8% for fresh; soil stored and indoor stored cleistothecia; respectively (Table 3). The significant differences among the methods of cleistothecia storage revealed that percentage of unviable cleistothecia were higher (73.2%) when kept in soil but it was reduced to 51% in case of indoor storage. Viable cleistothecia from the three categories used in the pathogenicity test showed different results in reproducing powdery mildew infection. Fresh cleistothecia reproduced powdery mildew on 60% of the disease-free plants exposed to inoculum followed by indoor stored cleistothecia inoculum (40%) and 20% for cleistothecia inoculum stored in soil.

Table (3): survival of cleistothecia as measured by visual assessment of viability and infectivity of ascospores.

Cleistothecia	Mean (%) of cleistothecia with asci and ascospores X		
	viable	Non-viable	Plants infected z
Fresh after harvest	70.8y a	29.5 c	3/5
Stored in soil	26.8 c	73.2 a	1/5
indoor stored	48.8 b	51.2 b	2/5

X Viable cleistothecia with ascospores had vacuolated cytoplasm and no oil droplets, and non-viable cleistothecia had degenerated asci or ascospores with dark cytoplasm and numerous oil droplets.

y Number followed by the same letter within a column are not significantly different according to Duncan's multiple range test (P=0.05).

z Number of plants that become infected from inocula of viable cleistothecia with asci and ascospores over the total number exposed to inoculum.

DISCUSSION

Sugar beet has been recently introduced to Egypt in 1980s to minimize the gap between production and consumption of sugar from sugarcane. In 1997, farmers started to cultivate and supply the new sugar factory belong to Dakahlia Sugar Company with their production of sugar beet crop from new cultivars imported from Europe (ARC, 1996). Problems incited by different pests started to appear in sugar beet fields as minor or major epidemic infection favored by suitable environment for both host and pathogen.

Powdery mildew disease in sugar beet fields increases rapidly especially in fields has no chemical control or good cultural practices. Experience gained from field inspection showed that date of powdery mildew appearance is rather detectable in most inspected fields annually. In growing areas at the Nile Delta Egypt, the disease was first appears about April 1st, but in some years it appears in the last week of March. The earlier disease appears, the more severe disease will be. Yield loss from the powdery mildew can be as high as 40% if not controlled especially in late sowing dates. The average of disease incidence still less than 10% approximately as personal estimation for early and late sowing dates. Some producers may consider it as minor disease but we think that the outbreak of the disease could occur because; 1) sugar beet is a new crop in this area and repeating cultivation may help in build up of pathogen population year after year. 2) Nowadays, researchers and sugar beet producers need to be aware of climate changes or global warming which has a potential impact on plant pathogen interaction in which some minor diseases became major epidemic.

E. betae is known as obligatory air borne fungus and can germinate on leaf surfaces over a wide range of temperatures and relative humidities, even 0% RH, but the optimum is at 25°C and 100% RH (Drandarevski, 1978). This accounts for the higher level of damage inflicted in areas with hot, dry climates of the world including Egypt (Ruppel, 1995 and Francis, 2002).

Observation on diseases symptoms revealed that initial symptoms appeared on plants at field edges first and diseases severity increased on older leaves more than young leaves. Fields are much larger than the plots used in the present experiments, so that more spores might be expected to be produced within the field, and hence a gradient would be observed further from the field edge.

Data indicated that significant differences in disease incidence and severity was observed between field edge and inside field, and would have a major effect on disease development within adjacent fields. This information is necessary for the design of management practices and in agreements with the findings of O'Hara & Brown 1998 on their study of powdery mildew movement on barley.

Polycyclic epidemics on annual crops are often initiated by inoculum from other sources, such as volunteer plants, or specialist dormant structures, such as cleistothecia (Wolfe & McDermott, 1994) The precise role of the sexual cycle in E. betae remains unclear, but it suggests the possibility of over winter survival and the development of new, and perhaps more virulent races of mildew (Byford and Bentley, 1976). In the present study, cleistothecia were showed in abundance on all sugar beet commercial cultivars in 2002 and 2003 growing seasons. Theses observation indicate that environmental factors in Egypt favor cleistothecium formation as reported Also, from North Africa, Mediterranean countries (Mamluk and Weltzien, 1973) and countries of the former Soviet Union (Cooke & Scott, 1993); in Europe, UK (Byford & Bentely 1976) and the USA, Nebraska (Harveson, 2004).

Based on field observation, the initiation and development of cleistothecia on sugar beet field in any year given suitable condition for disease development. In the present study, the dense aggregations of cleistothecia on leaves make them a potentially important source of inoculum. In addition, higher incidence of mature of viable mature cleistothecia were observed on infected plants, resting cleistothecia buried in soil during summer or 'indoor stored cleistothecia. These cleistothecia were able to reproduce mildew symptoms on healthy plants inside greenhouse under weather condition conducive to primary ascosporic infection. These results clearly show that cleistothecia of E. betae can be considered as important source of inocula for powdery mildew of sugar beet in Egypt. Prevalence and perennation of cleistothecia sustain the possibility of the development of new, and perhaps more virulent races of mildew (Byford & Bentley, 1976, Francis, 2002 and Harveson, 2004).

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إنتشار الأجسام الثمرية للفطر المسبب لمرض البياض الدقيقى فى بنجر السكر بمحافظة الدقهلية – مصر.

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ظهرت بدايات أعراض مرض البياض الدقيقى فى أواخر الشتاء وأوائل الصيف عادة فى حقول بنجر السكر بمحافظة الدقهلية. وعلى أساس هذه الملاحظات أجريت دراسة حقلية فى الفترة بين شهرى أبريل ومايو خلال الموسمين الزراعيين 2002 و 2003، وذلك لتقدير نسب وشدة الاصابة بالمرض على بعض الأصناف التجارية للبنجر. وأظهرت النتائج أن هناك زيادة تدريجية فى نسبة وشدة الاصابة بمرور الوقت منذ بداية ظهور المرض. ولوحظ أيضا أن هناك فروق معنوية بين نسب وشدة الأصابة على النباتات فى حواف وداخل الحقل. وفى نهاية شهر أبريل تكونت الأجسام الثمرية بغزارة على كل الأصناف التى تم فحصها خلال الموسمين 2002 و 2003 وأظهرت النتائج ان هناك فروق فى كثافة الأجسام الثمرية بين الأوراق الحديثة والمسنة وبين الأسطح العلوية والسفلية للأوراق. وتراوحت نسبة الاجسام الثمرية البالغة من 35- 55% فى الفحص المبكر للنباتات ووصلت إلى 85% فى الفحص المتأخر. أما نسب الأجسام الثمرية الحية كانت 70.8 ، 26.8 و 48.8% وذلك للمجموعات الثلاثة من الاجسام الثمرية الطازجة، والمخزنة فى التربة والمخزنة تحت ظروف جو الغرفة على التوالى. وأعطت هذه الأجسام الثمرية من المجاميع الثلاثة والتى استخدمت فى اختبار التطفل نسب مختلفة من أعراض المرض على النباتات السليمة التى عرضت للقاح الجراثيم الأسكية لها. وتوضح هذه النتائج بوضوح أن الأجسام الثمرية للفطر *E.betae* يمكن أن تعتبر مصدر هام للقاح الأولى لمرض البياض الدقيقى فى بنجر السكر فى مصر. وأن إنتشار وبقاء هذه الأجسام الثمرية من موسم لأخر قد يودى الى نشوء سلالات جديدة من الفطر ذات قدرة تطفلية أعلى.