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# Viability of Wheat Rust Urediniospores Produced on Different Stages of Susceptible Plants

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The longevity of freshly collected urediniospores of the wheat rust diseases, *i.e.* stem, leaf, and stripe rusts caused by *Puccinia graminis* f.sp. *tritici*, *P. triticina*, and *P. striiformis* f.sp. *tritici*, respectively, collected from different developed plant stages were studied. The varieties employed were Little club, Thatcher and *Triticum spelta* Saharensis, for stem, leaf and stripe rust, respectively. Spores were produced on plants at different growth stages, *i.e.* seedling, booting, and adult plant stages. The collected urediniospores were kept at temperatures between 5°C and 30°C at 5°C increments.

The results revealed that 10°C, 5°C and 5°C were most favorable for short term preservation for stem, leaf and stripe rust fungi, respectively. The spores remained viable for 26, 46 and 2 weeks for seedling, booting and adult stages for stem, leaf and stripe rust, respectively.

It is worth mentioning that no effect due to host age on the longevity of the spores was observed. Spores kept on dry leaves and collected from plants of the adult stage remained viable longer at the abovementioned temperatures than any other temperatures. Stripe rust urediniospores were more sensitive than the other two rusts. Stem and leaf rust urediniospores were the long lasting and can be kept viable from season to season for greenhouse study.

**Key words:** *Puccinia graminis* f.sp. *tritici*, *P. triticina*, *P. striiformis* f.sp. *tritici*, urediniospores and wheat rusts.

Wheat plants are liable to be attacked by stem, leaf and stripe rust diseases under Egyptian conditions. Urediniospores are considered the most economically important in maintaining disease from season to season. Collecting rust samples for the three rusts, is the first step in wheat breeding program for rust resistance. These samples should be collected from different genotypes and from different locations to obtain all possible races to be included in rust nurseries. Rust collections are usually kept from season to another for race identification. In this respect, two main methods are usually followed. The first is a short-term preservation including dry leaf and glass tube methods (Leathers, 1961 and Eversmeyer and Burleigh, 1968) while, the second is a long-term preservation including liquid nitrogen and/or lyophilization method (Sharp and Smith, 1957; Loegering *et al.*, 1961 and Loegering and Harmon, 1962).

Generally, the longevity of rust urediniospores is affected by many factors. Temperature is considered the main factor which affects urediniospores longevity (Hung and Chien, 1961; Eversmeyer and Burleigh, 1968; Osman and Manners, 1983; Dennis, 1987; Eversmeyer and Karmer 1994 and 1995 and Nazim *et al.*, 2003).

Spores kept on dry leaves of the susceptible genotypes survived longer than spores kept in glass tubes (Nazim *et al.*, 2003). However, no data were available to describe the effect of host growth stage on the longevity of rust urediniospores. Therefore, the main objective of this study was to determine the effect of host age or host growth stage on spore viability for spores kept on dry leaves, under different temperatures.

## Materials and Methods

This study was concerned with the viability of urediniospores of rust diseases, *i.e.* stem rust (*Puccinia graminis* f.sp. *tritici*), leaf rust (*P. triticina*), and stripe rust (*P. striiformis* f.sp. *tritici*).

The urediniospore viability was studied under the effect of host growth stage by storing them on dry leaves or in glass tubes under different temperatures ranging from 5°C to 30°C with 5°C intervals.

*Plant materials:*

The wheat genotypes, *i.e.* Little club, Thatcher and *Triticum spelta* Saharensis, were used for stem, leaf and stripe rust, respectively. Seeds of each were sown at three dates with one month intervals to get three growth stages at the same time. These stages were seedling, booting, and adult stages.

*Rust materials:*

Rust races were obtained from the Wheat Dis. Dept., Plant Pathol. Res. Instit., Agric. Res. Centre, Egypt. Leaf and stem rust races were obtained from the Rust Laboratory at Giza and the stripe rust race was obtained from the Rust Laboratory at Sakha Agric. Res. Station, ARC.

The rust races used in this study were race TSK (stem rust), race KSPBS (leaf rust) and race 499 E-95 (stripe rust).

*Rust multiplication:*

Urediniospores of stem rust race (TSK) were multiplied on seven-day-old seedlings of the susceptible wheat variety Little club, while leaf and stripe rust races were increased on the two varieties, *i.e.* Thatcher and *Triticum spelta* Saharensis, respectively. In all cases, the plants were re-inoculated several times and the spores were collected by shaking the rusted leaves in a glass tube and kept, after drying at room temperature, in the refrigerator until used.

*Rust inoculation:*

*a) At seedling stage:*

Seven day old seedlings of the susceptible genotypes were sprayed with distilled water and gently rubbed by moistened fingers to scratch the wax layer and

transferred to a humid chamber. The plants were then inoculated with the freshly collected urediniospores using a baby cyclone (Tervet and Cassel, 1951).

*b) At adult stage:*

Adult plant of 60-70 days old of the susceptible genotypes were sprayed with distilled water and rubbed with moistened fingers. Plants were then sprayed with water in incubation chambers (15-20°C and 100% humidity) and then inoculated by dusting them with a mixture of urediniospores and talcum powder at the ratio of 1:5 (v/v).

*c) Incubation:*

The inoculated plants were incubated overnight to allow the rust spores to germinate and cause infection. The inoculated plants were then transferred onto the benches in the green house under natural conditions.

*d) Testing of spore viability:*

Plants of different growth stages were inoculated with urediniospores of the previously mentioned races. Infected leaves were collected, left overnight at room temperature (20-25°C) to reduce the humidity of the samples and placed in glassine end opening envelopes about 9 x 17cm before storing.

When the rust pustules were fully developed, the spores were collected by shaking leaves of infected plants onto a watch glass and were then transferred into vials. They were stored at different temperatures from 5-30°C.

Urediniospores germinability was the only measure used to test viability. It was determined by shaking the infected stored leaves on 2% water agar medium and then incubating for 24 hr at 18±2°C. About 100 spores of each race in three microscopic fields were checked to determine the percentage of germination (Chester, 1946). The germination test was carried out immediately after collection and then at seven days intervals. When the germination test indicated that the urediniospores were unable to germinate, three more germination tests were carried out to ensure the non-viability of the urediniospores. Re-inoculation of the susceptible plants was carried out with the non-viable urediniospores to confirm the non-viability.

## Results

*a) Viability of stem rust urediniospores:*

The results obtained revealed that, the viability of the spores when measured as percentage of germination, at zero time (immediately after urediniospores collection) was 100% for stem rust spores collected from the three growth stages.

*1- Seedling stage:*

Fig. 1 shows that urediniospores stored at 5°C retained 100% viability for 3 weeks and sharply declined up to 8$^{th}$ week. Thereafter, it slightly decreased up to the 18$^{th}$ week. After 26 weeks no viable spores were recorded at this temperature. Also, urediniospores stored at 10°C remained viable (100%) for four weeks and then viability slightly declined up to the 19$^{th}$ week. After this period of storage, the viability sharply decreased to zero after 26 weeks.

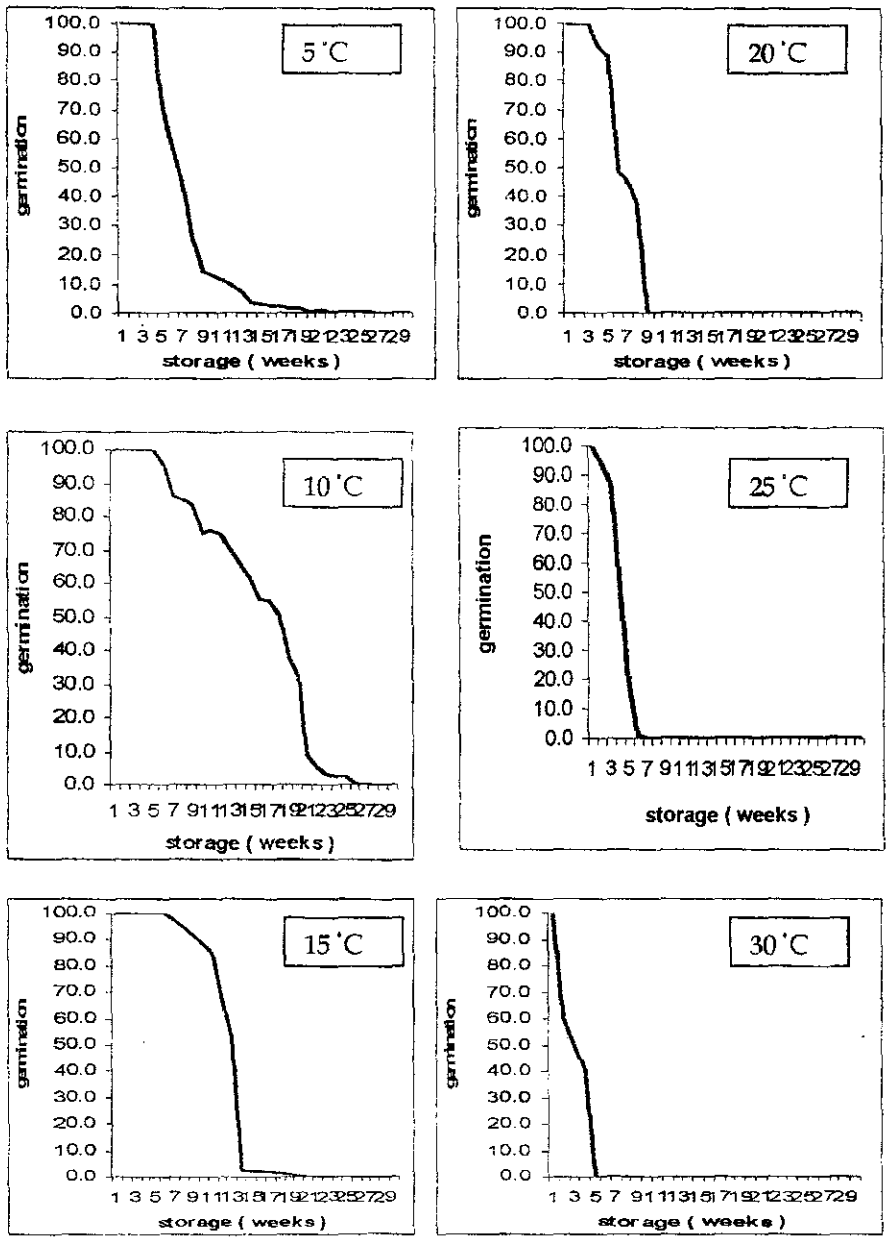


**Fig. 1. Percentage of urediniospore germination of stem rust (*Puccinia graminis* f.sp. *tritici*) stored at different temperatures (5-30°C) on leaves of cv. Little club at seedling stage.**

On the other hand, the spores remained viable (100%) at 15°C for five weeks and the viability slightly decreased up to the 12th weeks. After 12 weeks spore viability was clearly decreased and lost after 19 weeks.

At 20°C, the stored urediniospores retained 100% viability for three weeks and this then declined slightly up to the 7th week. Therefore, viability has sharply decreased and was completely lost after 9 weeks. While at 25°C, the percentage germination was 93.7% in the first week and their viability slightly declined up to the 3rd week. After three weeks, the viability sharply decreased until it was completely lost after 5 weeks (Fig. 1).

At 30°C, the percentage of germination was 78.2% in the first week and their viability slightly declined up to 2nd week. After two weeks, the viability sharply decreased until it was completely lost after 4 weeks (Fig. 1).

*2. Booting stage:*

Data shown in Fig. (2) indicate that the percentage of germination was 100% for three weeks for urediniospores stored at 5°C. Viability then decreased sharply up to the 5th week. After 5 weeks, viability has gradually declined until it was lost after 18 weeks, while, spores stored at 10° C showed 100% germination for five weeks. Viability then decreased gradually up to 19th week. Thereafter, viability has sharply decreased until spores lost their viability after 26 weeks (Fig. 2).

Urediniospores stored at 15°C retained 100% viability for five weeks then gradually decreased until it was lost after 18 weeks. On the other hand, urediniospores kept at 20°C retained 100% viability for three weeks then decreased gradually up to 7 weeks until it was lost after 9 weeks (Fig. 2).

At 25°C, the percentage of germination was 94.1% for only one week then gradually decreased to zero percent after 6 weeks, but at 30°C the percentage germination was 90.9% in the first week. The spores then lost their viability after 7 weeks.

*3. Adult stage:*

Data shown in Fig.3 indicate that the urediniospores stored at 5° C retained 100% viability for three weeks then sharply decreased up to the 4th week. After 21 weeks, no germination was observed, while, urediniospores stored at 10°C showed 100% viability for 8 weeks and then declined up to the 19th week. After 19 weeks the viability has sharply decreased as spores lost their viability after 27 weeks.

Urediniospores stored at 15°C remained 100% viable spores for 7 weeks and viability then declined up to the 12th week. After 12 weeks the viability sharply decreased until it was lost after 19 weeks.

Urediniospores stored at 20°C retained 100% viable for three weeks then decreased slightly up to the 7th week. Therefore, the viability has sharply decreased and no viable spores were recorded after 10 weeks of storage.

At 25°C the spores retained 100% viability for only one week. Viability has then declined up to the 4th week and was zero after 7 weeks.

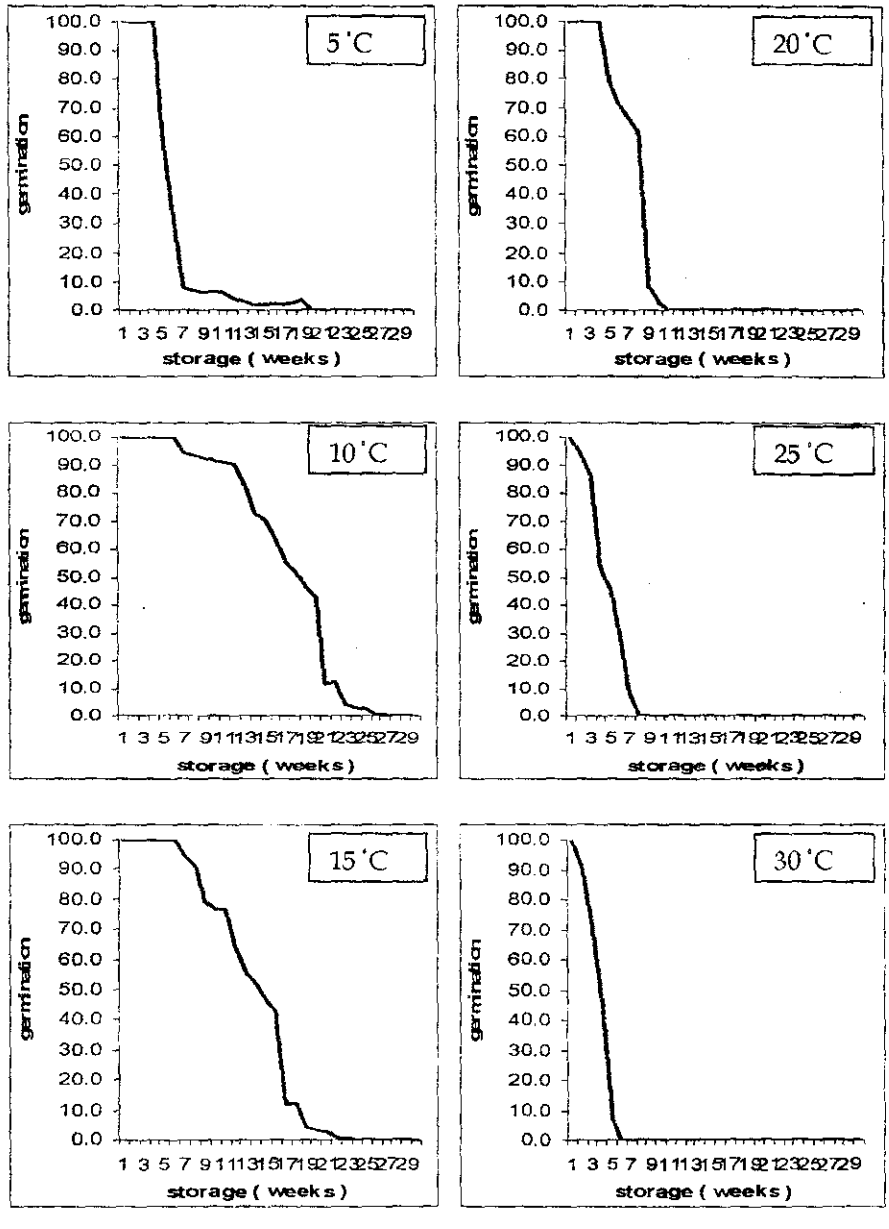


**Fig. 2. Percentage of urediniospore germination of stem rust (*Puccinia graminis*) stored at different temperatures (5-30°C) on leaves of cv. Little club at booting stage.**

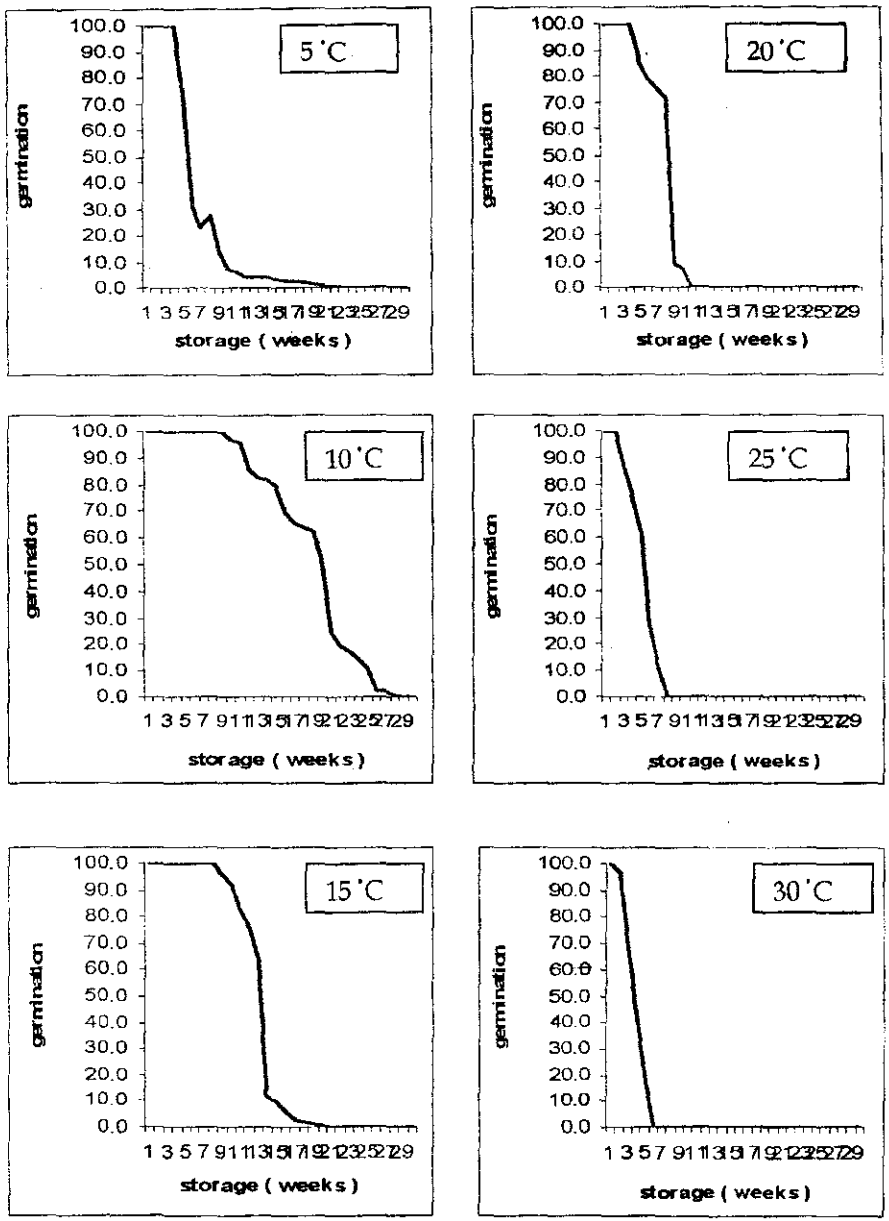


**Fig. 3. Percentage of urediniospore germination of stem rust (*Puccinia graminis* f.sp. *tritici*) stored at different temperatures (5-30°C) on leaves of cv. Little club at adult stage.**

At 30°C spores germination was 96.7% in the first week. Viability was lost entirely after four weeks.

Data presented in Table 1 show that spores kept at 10°C remained viable for 26 weeks at the seedling and booting stages and for 27 weeks for the adult stage. Longevity of the spores decreased when the temperature was varied by more than 10°C. No effect of the host age on the longevity of spores was observed. Spore viability decreased by increasing temperature.

**Table 1. Effect of growth stage of wheat plants on stem rust urediniospore viability stored at different temperatures on dry leaves of cv. Little club**

| Host age | Storage temperature / longevity (week) | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
| Seedling | 26 | 26 | 19 | 9 | 5 | 4 |
| Booting | 18 | 26 | 18 | 9 | 6 | 4 |
| Adult | 21 | 27 | 19 | 9 | 6 | 4 |

Generally, spores kept on dry leaves and collected from plants of the adult stage remained viable longer at 10°C than at any other temperature under the study. In addition, percentage of spore germination was higher at 10°C than for the other treatments (Figs. 1, 2 and 3).

*b. Viability of leaf rust urediniospores:*

*1. Seedling stage:*

Results in Fig. (4) show that the urediniospores at 5°C retained 100% viable for 7 weeks. Thereafter, viability decreased slightly up to the 19th week, then decreased and completely lost after 46 weeks. Whereas, urediniospores stored at 10°C remained 100% viable for 8 weeks and thereafter, the viability decreased slowly until 19th week, then the viability has sharply decreased until it was lost after 29 weeks.

Urediniospores kept at 15°C showed 100% germination for seven weeks, and then germination percentage gradually decreased up to the 18th week. No germinated spores were found after 19 week.

At 20°C the spores retained 100% viability for only two weeks, and then gradually decreased up to the 7th week. Viability dropped suddenly in the 8th week, and was lost entirely in the 9th week.

At 25°C, the percentage of germination was 85.6% in the first week and viability then decreased sharply until it was completely lost after 6 weeks.

At 30°C, the percentage germination was 58.3% in the first week and fell to zero after three weeks.

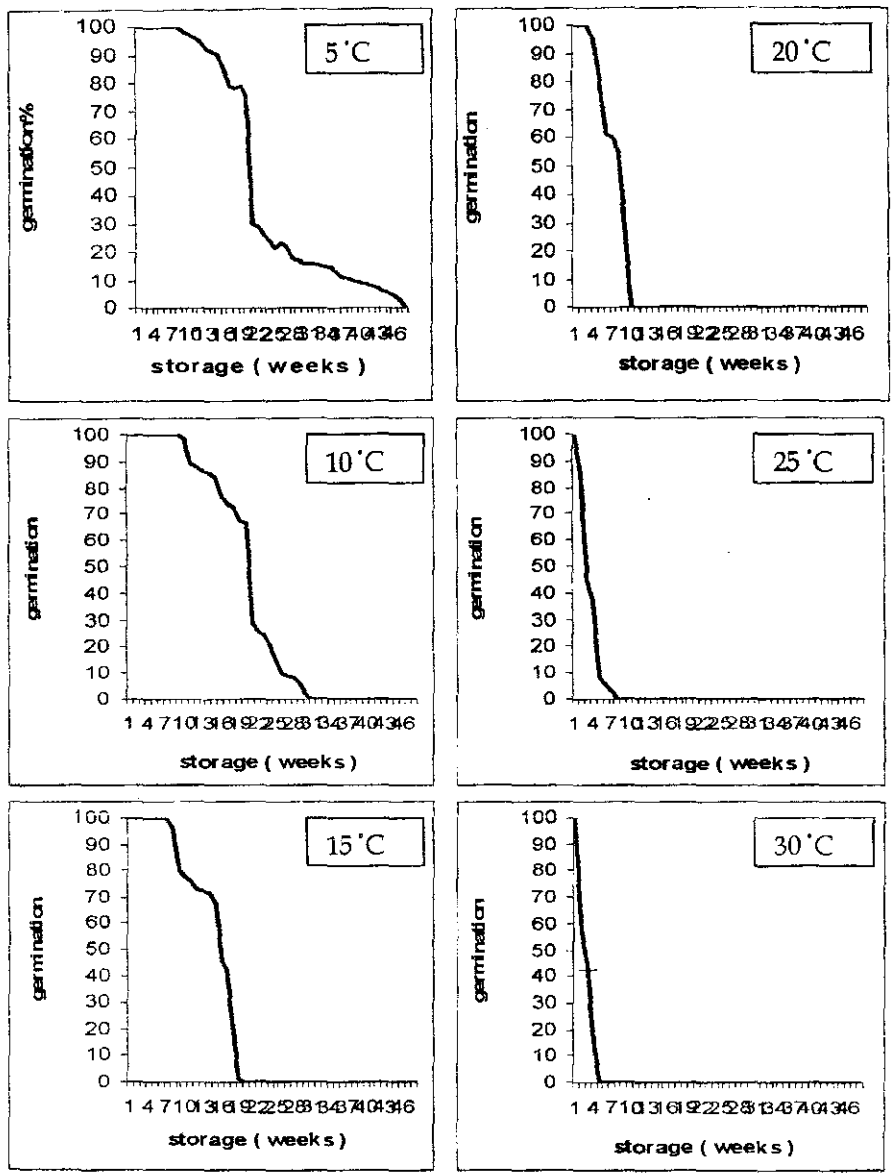


**Fig. 4. Percentage of urediniospore germination of leaf rust (*Puccinia triticina*) stored at different temperatures (5-30°C) on leaves of cv. Thatcher at seedling stage.**

*2. Booting stage:*

Results in Fig. (5) show that the urediniospores retained 100% viability for seven weeks and thereafter viability decreased slightly up to the 19th week. After 19 weeks viability sharply decreased until it was lost completely after 46 weeks.

The stored urediniospores retained 100% viability for eight weeks. Almost half of the spores were still viable until the 19th week. After 19 weeks the viability sharply decreased until it was completely lost after 32 weeks.

The urediniospores stored at 15°C retained 100% viability for three weeks and viability then decreased slightly up to the 17th week. After 17 weeks the viability decreased sharply and was zero after 18 weeks.

The stored urediniospores at 20°C showed 100% germination for three weeks and this then sharply decreased. It was completely lost after 9 weeks. At 25°C the percentage of germination was 77.2% in the first week and the spores lost their viability entirely after 3 weeks.

At 30°C the percentage of germination was 67.0% in the first week and viability was completely lost after 3 weeks.

*3. Adult stage:*

Data illustrated by Fig. (6) show that the urediniospores retained 100% viability for 7 weeks and then decreased slightly up to the 23rd week. After 23 weeks the viability has sharply decreased and lost after 46 weeks.

At 10°C, the spores remained 100% viable for 9 weeks. Viability then decreased slowly to zero after 37 weeks.

At 15°C, the spores retained 100% viability for 5 weeks and thereafter, viability decreased gradually up to the 17th week. After 17 weeks viability decreased sharply and was completely lost after 19 weeks.

At 20°C, the spores retained 100% viability for 3 weeks and viability then declined gradually up to the 7th week. After 7 weeks the viability decreased sharply and was lost completely after 9 weeks.

At 25°C, the viability was 96.2% in the first week and then dropped from 46.0% to 12.0% after 3 weeks followed by a slight decline up to the 7th week.

At 30°C, the viability was 95.2% in the 1st week and viability then sharply decreased from 71.1% to 20.0% after 2 weeks until the spores lost their viability after 3 weeks.

Data presented in Table (2) indicate that the best temperature for urediniospores preservation was 5°C. In all cases the longevity of the spores decreased gradually by increasing the storage temperatures from 46 weeks at 5°C to 2-3 weeks at 30°C.

Generally, spores developed on booting and adult plants and kept at 10°C remained viable longer with 3 weeks than those developed on seedlings.

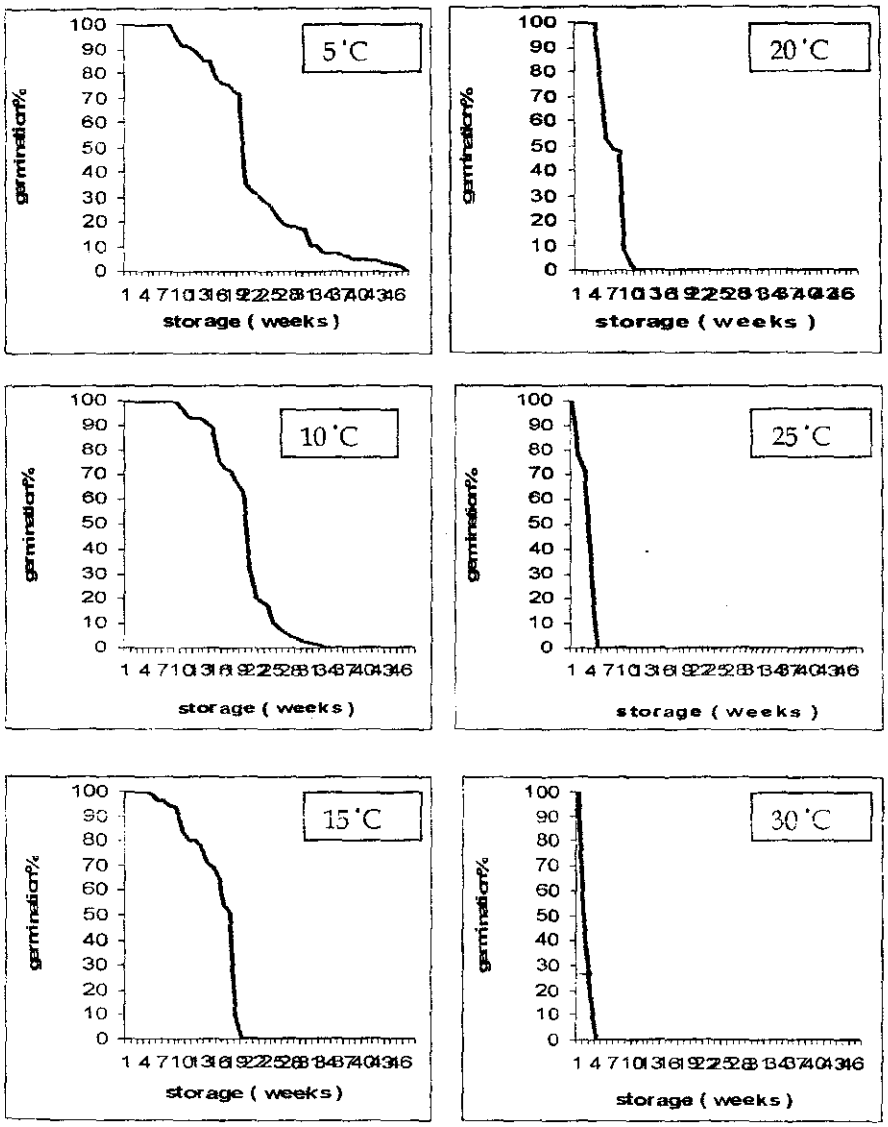


**Fig. 5. Percentage of urediniospore germination of leaf rust (*Puccinia triticina*) stored at different temperatures (5-30°C) on leaves of cv. Thatcher at booting stage.**

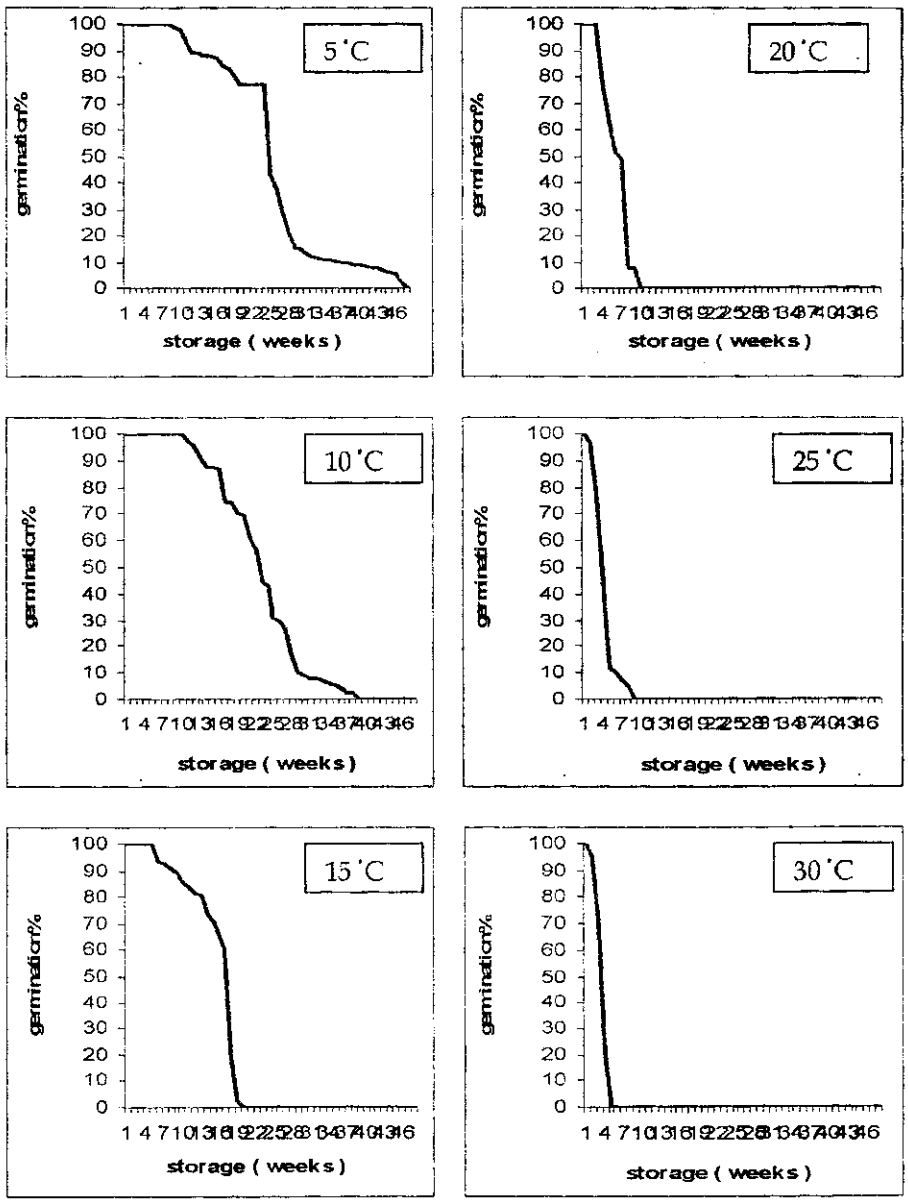


**Fig. 6. Percentage of urediniospore germination of leaf rust (*Puccinia triticina*) stored at different temperatures (5-30°C) on leaves of cv. Thatcher at adult stage.**

**Table 2. Effect of growth stage of wheat plants on leaf rust urediniospore viability stored at different temperatures on dry leaves of cv. Thatcher**

| Host age | Storage temperature / longevity (week) | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
| Seedling | 46 | 29 | 19 | 8 | 6 | 2 |
| Booting | 46 | 32 | 17 | 9 | 3 | 3 |
| Adult | 46 | 32 | 19 | 9 | 4 | 3 |

*c. Viability of yellow rust urediniospores:*

*1. Seedling stage:*

Data presented in Table (3) show that the urediniospores at 5°C viability was 11.2% for the 1st week and 0.6% for the 2nd week. After 3 weeks the urediniospores viability was zero. While, at 10°C, the spore viability was 5.7% in the 1st week and then zero after that. At 15°C, the spores viability was 1.6% in the 1st week, then the spores lost their viability entirely. No germination was observed at 20, 25 and 30°C.

**Table 3. Percentage of stripe rust (*Puccinia striiformis*) urediniospores germination stored at different temperatures (5-30°C) and developed on var. *Triticum spelta* Saharensis at seedling stage**

| Storage period (weeks) | Storage temperature / spore germination (%) | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
| 0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 |
| 1 | 11.2 | 5.7 | 1.6 | 0.0 | 0.0 | 0.0 |
| 2 | 0.6 | 0.0 | 0.0 | 0.0 | | |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | | |
| 4 | 0.0 | 0.0 | 0.0 | | | |
| 5 | 0.0 | | | | | |

*2. Booting stage:*

Data presented in Table (4) show that the urediniospores viability was 12.8% in the first week and dropped to 0.4% in the 2nd week and after 2 weeks was zero. At 10°C, the spore viability was 6.2% in the first week and zero after 1 week.

At 15°C, the spore viability was 2.0% in the 1st week and zero after 1 week. At 20°C, the spores viability was 1.9% in the 1st week and zero after 1 week. No germination was recorded at temperatures of 25 and 30°C.

**Table 4. Percentage of stripe rust (*Puccinia striiformis*) urediniospores germination stored at different temperatures (5-30°C) and developed on var. *Triticum spelta* Saharensis at booting stage**

| Storage period (weeks) | Storage temperature / spore germination (%) | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
| 0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 |
| 1 | 12.8 | 6.2 | 2.0 | 1.9 | 0.0 | 0.0 |
| 2 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| 5 | 0.0 | 0.0 | 0.0 | 0.0 | | |

*3. Adult stage:*

At 5°C, results shown in Table (5) indicated that urediniospores viability was 22.4% in the first week, dropped to 1.3% in the 2nd week and was zero after 2 weeks.

At 10°C, the percentage of germination was 5.7% in the first week and zero after the 1st week. At 15°C, the spore viability was 5.7% in the 1st week and zero after 1 week. At 20°C, the spore viability was 1.6% in the 1st week zero after 1 week.

No germination was observed at temperatures 25 and 30°C. Generally, results shown in Tables (3, 4 and 5) indicate that low percentage of germination of urediniospores of *P. striiformis* and short period of longevity were recorded for all growth stages which ranging from 1-2 weeks at 5°C and 10°C. It was one week for all cases except at 5°C for spores developed on the adult stage (Table 6).

It was also found that germination of stripe rust urediniospores was very low even when spores were tested after their release (Tables 3, 4 and 5).

**Table 5. Percentage of stripe rust (*Puccinia striiformis*) urediniospore germination stored at different temperatures (5-30°C) and developed on var. *Triticum spelta* Saharensis at adult stage**

| Storage period (weeks) | Storage temperature / Spore germination (%) | | | | | |
|---|---|---|---|---|---|---|
| | 5° C | 10° C | 15° C | 20° C | 25° C | 30° C |
| 0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 | 72.0 |
| 1 | 22.4 | 5.7 | 5.7 | 1.6 | 0.0 | 0.0 |
| 2 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 | 0.0 | | | |
| 5 | 0.0 | | | | | |

**Table 6. Effect of growth stage of wheat plants on urediniospore viability of stripe rust stored at different temperatures, on dry leaves of var. *Triticum spelta* Saharensis**

| Host age | Storage temperature / longevity (week) | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
| Seedling | 2 | 1 | 1 | 0 | 0 | 0 |
| Booting | 2 | 1 | 1 | 1 | 0 | 0 |
| Adult | 2 | 1 | 1 | 1 | 0 | 0 |

## Discussion

Rust diseases of wheat appear wherever wheat plants are grown in Egypt. They usually appear at flowering and/or the following stages, causing considerable losses in grain yield. (Nazim *et al.*, 1983 and El-Daoudi *et al.*, 1996). The urediniospores, in particular, comprise the dangerous stage to wheat, although they are short lived in nature (Burleigh *et al.*, 1969).

In breeding programs for rust resistance, preservation of viable urediniospores is a very essential step. This program starts by collecting infected materials from different locations and from different wheat genotypes for each rust of wheat (Stakman and Harrar, 1957). Rust samples can be collected as infected leaves or as urediniospores. In both cases viable spores should be preserved for virulence survey or for testing generations or wheat genotypes in rust nurseries.

Generally, two main methods for spore preservation are known. The first is a short-term preservation, including the dry leaf method and the glass tube method, while the second is a long-term preservation in liquid nitrogen and by lyophilization.

Spores can be kept for several years in liquid nitrogen (Flor, 1954; Loegering and Harmon, 1962; Bromfield, 1964 and Loegering *et al*, 1961). Lyophilization is a process by which spores stored in glass tubes can be held at room temperature for several months without loss viability, while lyophilized spores remain viable for several years when spores are kept at low temperatures (Sharp and Smith, 1957).

In the course of this study, short-term preservation was undertaken for spores kept on dry leaves for stem, leaf and stripe rust diseases. Host age, as a factor affecting spore viability was studied at different temperatures between 5-30°C with 5°C intervals.

In this study, the effect of three growth stages, *i.e.* seedling, booting and adult stages on urediniospores viability was studied. The highly susceptible varieties, *i.e.* Little club, Thatcher and *Triticum spelta* Saharensis were used for stem, leaf and stripe rusts, respectively.

Viability of spores was tested by determining their germinability on water agar after 24 hours incubation. Tests of germination were carried out every 7 days until germination reached 0%.

Plants of the different stages were inoculated with the previously mentioned races. After the rust was fully developed, infected leaves and fresh urediniospores were collected and stored at the different temperatures (5-30°C) as mentioned before. The percentage of germination was determined every week. The results obtained revealed that, the percentage of germination of the urediniospores of the rusts was higher on the dry leaves of the adult stage than booting and/or seedling stages. This confirms that, keeping spores on dry leaves of the adult plants allows longer survival than storing them on the booting and/or seedling stages.

Spores of stem rust survived 27 weeks on leaves of adult plants at 10°C, whereas leaf rust spores survived 46 weeks on leaves of adult plants at 5°C.

On the other hand, stripe rust spores survived for a shorter period than the other two rusts. Stripe rust urediniospores were more sensitive than the other two rust diseases. Therefore, they cannot be kept for longer than two weeks whatever the storage temperature is (5-30°C).

However, for short-term preservation spores can be kept on dry leaves at low temperature. Choosing the proper stage of plant growth, on which spores are kept, is very important in this experiment

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# حيوية الجراثيم اليوريدية لأصداء القمح والناتجة على أطوار نمو النبات المصاب المختلفة

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تمثل عمليه جمع عينات الجراثيم اليوريديه (الطور المعدي للقمح) لأصداء القمح الثلاثة (الصدأ الأصفر- صدأ الأوراق- صدأ الساق) المرحلة الأولي سواء في برنامج التربية للمقاومة للأصداء أو عند دراسة التنوع او التغير في أعداد الكائن الممرض. وتمثل عمليه حفظ الجراثيم اليوريديه من موسم لاخر مشكله حيث تفقد تلك الجراثيم حيويتها وبسرعة عند التخزين الغير جيد. لذا تهدف هذه الدراسة إلى تحديد انسب درجات الحرارة والتي يمكن عندها حفظ الجراثيم اليوريديه للأصداء الثلاثة والناتجة على أطوار نمو مختلفة من العائل الحساس للاصابه.

استخدمت في هذه التجربة أصناف قمح عالية القابلية للإصابة وهي الصنف Little Club لصدأ الساق الأسود، والصنف Thatcher لصدأ الأوراق، والصنف *Triticum spelta* للصدأ الأصفر حيث أجريت عملية العدوى للنباتات وذلك لأطوار النمو المختلفة لكل صنف على حده وهي البادرة (Scedling stage) والطور المغمد (Booting stage) وطور النبات البالغ Adult stage ، وقد أجريت العدوى في نفس الوقت للأعمار الثلاثة والتي تم الحصول عليها بالزراعة في مواعيد مختلفة.

ثم جمعت العينات المصابة والجراثيم بالطريقة التي أشرت إليها أنفا ثم حفظت على نفس درجات الحرارة (5-30°م) بفاصل خمس درجات مئوية ، ثم تم تقدير نسبة الإنبات على بيئة الأجار المائي وذلك مرة كل أسبوع وقد أوضحت النتائج الآتي :

1- أرتفاع نسبة الإنبات لجراثيم الأصداء الثلاثة المأخوذة في طور البلوغ المحفوظة على أوراق النبات الجافة .
2- احتفظت جراثيم الأصداء الثلاثة - المخزنة على أوراق النباتات في طور البلوغ - بحيويتها فترة أطول عنها في الطورين: المغمد و البادرة.
3- وكان الصدأ الأصفر هو أكثر الأصداء حساسية عن كل من الصدأ البرتقالي أو صدأ الساق.
4- كما كانت فترة بقاء الجراثيم - المحفوظة على أوراق النبات الجافة - محتفظة بحيويتها أطول منها في حالة الجراثيم المحفوظة داخل أنابيب الاختبار بفترة تصل إلى حوالي أسبوع تقريباً.
5- تقل فترة احتفاظ الجراثيم بحيويتها بارتفاع درجات الحرارة بوجه عام للأصداء الثلاثة في مختلف أطوار النمو.

ففي حالة مرض صدأ الساق ظلت الجراثيم محتفظة بحيويتها لمدة 27 أسبوع وذلك عندما حفظت على درجة حرارة 10°م على أوراق النبات البالغ الجافة وقد انخفضت إلى 26 أسبوع في حالة الطورين المغمد والبادرة على أوراق النبات في نفس درجة الحرارة

أما في حالة مرض صدأ الأوراق فكانت أفضل درجة حرارة للحفظ هي 5°م حيث احتفظت الجراثيم بحيويتها لمدة 46 أسبوع عندما حفظت على الأوراق الجافة للنبات في أطوار نموه الثلاثة

وعموما فان أطول فترة لبقاء الجراثيم محتفظة بحيويتها كانت 27 أسبوع على درجة حرارة 10°م في حالة صدأ الساق أما في حالة صدأ الأوراق فكانت 46 أسبوع على درجة حرارة 5°م ولم تتعد أسبوعين في حالة الصدأ الأصفر على درجة 5°م.

لذا فانه لا ينصح بحفظ جراثيم مرض الصدأ الأصفر بالطرق العادية حيث إنها لا تبقى أكثر من أسبوعين مع وجود فروق طفيفة بين الحفظ على أوراق النبات الجافة والحفظ في أنابيب زجاجية، وإنما ينصح باستخدام طرق أخرى.