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Résumé de l'article

Afin de mieux connaître les facteurs qui influencent leur pouvoir pathogène, nous avons recherché l'activité glaçogène chez trois espèces de *Fusarium* : le *Fusarium oxysporum*, le *F sporotrichioides* et le *F tricinctum*. Le *F acuminatum* et le *F avenaceum* ont servi de témoins positifs (lna+). La recherche de l'activité glaçogène a été effectuée à l'aide d'un test rapide et simple de nucleation en éprouvettes. Douze des 42 isolats testés du *F oxysporum* et 8 des 14 isolats testés du *F tricinctum* se sont révélés positifs alors que les isolats du *F sporotrichioides* furent négatifs. La température de nucleation tendait à augmenter avec le temps, pouvant atteindre -1°C, température maximale rapportée pour les bactéries et beaucoup plus élevée que la température maximale connue pour les champignons et pour les algues et les cyanobactéries de lichen en culture pure. C'est la première fois qu'une activité glaçogène est démontrée pour le *F oxysporum*.

*Communication brève / Short Communication*

# Ice nucleation activity identified in some phytopathogenic *Fusarium* species

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In order to know which species of *Fusarium* are ice nucleating and to determine the factors affecting their pathogenicity, ice nucleation activity (INA) was examined in *Fusarium oxysporum, F. sporotrichioides*, and *F. tricinctum*. Positive controls (Ina+) used were *F. acuminatum* and *F. avenaceum*. The test for fungal INA was done with a simple and rapid tube nucleation assay. Twelve out of the 42 *F. oxysporum* isolates, and 8 out of 14 *F. tricinctum* isolates were Ina+. No INA was detected in *F. sporotrichioides*. In this test, the threshold freezing temperature tended to increase with culture age, reaching a peak of -1°C in a few samples, which is as high as the warmest INA reported for bacteria, and higher than the INA detected in pure cultures of free-living fungi, lichen fungi, lichen algae and cyanobacteria. This is the first report of INA for *F. oxysporum*.

**Richard, C., J.-G. Martin, and S. Pouleur. 1996. Ice nucleation activity identified in some phytopathogenic *Fusarium* species. PHYTOPROTECTION 77 : 83-92.**

Afin de mieux connaître les facteurs qui influencent leur pouvoir pathogène, nous avons recherché l'activité glaçogène chez trois espèces de *Fusarium* : le *Fusarium oxysporum*, le *F. sporotrichioides* et le *F. tricinctum*. Le *F. acuminatum* et le *F. avenaceum* ont servi de témoins positifs (Ina+). La recherche de l'activité glaçogène a été effectuée à l'aide d'un test rapide et simple de nucléation en éprouvettes. Douze des 42 isolats testés du *F. oxysporum* et 8 des 14 isolats testés du *F. tricinctum* se sont révélés positifs alors que les isolats du *F. sporotrichioides* furent négatifs. La température de nucléation tendait à augmenter avec le temps, pouvant atteindre -1°C, température maximale rapportée pour les bactéries et beaucoup plus élevée que la température maximale connue pour les champignons et pour les algues et les cyanobactéries de lichen en culture pure. C'est la première fois qu'une activité glaçogène est démontrée pour le *F. oxysporum*.

Ice nucleation is the initiation of the crystallization of subcooled water (below 0°C). Ice nucleation activity (INA) at temperatures above -5°C has been known in bacteria since 1976 (Vali *et al.* 1976). Recently, INA was associated with lichens (Kieft 1988) and with free-living fungi (Pouleur *et al.* 1992; Tsumuki *et al.* 1992). Until now, non-lichen fungal INA has been known only in the genus *Fusarium* (Ashworth and Kieft 1995). Two species of *Fusarium* have been reported as ice-nucleating (Ina+) fungi : *F. acuminatum* Ellis & Everh. (Pouleur *et al.* 1992) and *F. avenaceum* (Fr. : Fr.) Sacc. (Hasegawa *et al.* 1994; Pouleur *et al.* 1992; Sychrova

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*et al.* 1994). INA initiated at relatively high temperature by these pathogenic fungi may give them an advantage for root invasion. Richard *et al.* (1982, 1985) and Smith and Olien (1978) showed that infection of plant roots by fusaria is enhanced by freezing. It is thus interesting to know which species of *Fusarium* are Ina+ in order to better understand the factors affecting their pathogenicity and to check the validity of this criteria for use in the identification of Ina+ *Fusarium* species.

INA was investigated in three species of *Fusarium*, which are well known plant pathogens and common soil inhabitants (Farr *et al.* 1989) : *F. oxysporum* Schlechtend. : Fr. (42 isolates), *F. sporotrichioides* Sherb. (14 isolates) and *F. tricinctum* (Corda) Sacc. (14 isolates). Two isolates of both *F. acuminatum* and *F. avenaceum* were used as Ina+ controls. Two cultures (replicates) of each isolate were tested. The test for fungal INA was done by using a tube nucleation test (Pouleur *et al.* 1992). Instead of scraping off the mycelial mat from the surface of the agar, a mycelium plug (5 mm diam) was recovered from the oldest part of the colony (near the centre). The plug was dropped in a small test tube (13 × 100 mm) containing 2 mL sterile deionized water, subcooled at -5.0°C in a Lauda refrigerating circulator bath (model RC-6; Brinkman Instrument Co., Rexdale, Ontario, Canada) and free of nuclei according to a test at -10.0°C for 1 h. Freezing of the tube contents within 10 min of dropping the plug was recorded as positive (Ina+) for that species. The cultures were tested after 7, 14, 21 and 28 d of growth. Following all the nucleation tests, the tubes and their contents were allowed to warm to room temperature. After 20 min, they were put back in the bath for 20 min and cooled down to 0°C. Afterwards the temperature was lowered at the rate of 0.1°C $min^{-1}$ until it reached -10.0°C. The temperature at which the contents of the tubes froze (the threshold temperature) was recorded.

After 7 d, and up to 28 d, both isolates of *F. acuminatum* and *F. avenaceum*, the Ina+ controls, were positive (Table 1). Out of 43 *F. oxysporum* isolates, 12 were Ina+. Isolates nos. 301, 709, 710 and 712 were the most consistent over time and replicates, and may definitively be regarded as Ina+. The other ones did not react consistently. It is not surprising that some strains of an Ina+ species were Ina-; in bacteria, not all strains within a species are Ina+ (Hirano *et al.* 1978; Paulin and Luisetti 1978). In a previous survey of INA in *Fusarium* species, no Ina+ isolates of *F. oxysporum* had been found (Pouleur *et al.* 1992).

No INA was detected in *F. sporotrichioides* except in two instances: at 7 d for isolate 538 sample B and at 21 d for isolate 609 sample B. The observed threshold temperatures, -8.2°C and -10°C for 538 and 609B, respectively, are too low to be considered Ina+.

Of the 14 *F. tricinctum* isolates tested, 9 were Ina+; 7 of these were consistently Ina+. Isolates 663 and 689 were positive at 14 and 21 d respectively, but did not exhibit a threshold temperature higher than -5.5°C. The maximum temperature at which INA was reported for *F. tricinctum* was -5.6°C (Sychrova *et al.* 1994). This is not recognized as Type 1 INA or warm INA temperature, *i.e.* nuclei active at temperatures above -5.0°C (Phelps *et al.* 1986). The nucleation temperatures of the Ina+ isolates of *F. tricinctum* in our test were usually higher than -5.0°C with a peak at -1.0°C at 21 d for isolate 666A. This confirms that *F. tricinctum* is Ina+ and indicates that it probably produces Type I nuclei.

When an Ina+ isolate was consistent over time and replicates, it usually showed a positive reaction as soon as 7 d and at all sampling periods. The threshold temperature tended to increase with time in most Ina+ isolates (Nos. 116, 368, 250, 301, 709, 710, 662, 665, 666, 684, 685, 686, 687 and 689), reaching a peak of -1°C in some instances. This temperature is as high as the warmest INA reported for bacteria, and higher than the INA detected in pure cultures of free-living fungi, lichen fungi, lichen algae and cyanobacteria as compiled by Ashworth and Kieft (1995). The highest INA previously reported is -1.9°C for the lichen mycobiont *Rhizoplaca chrysoleuca* (Sm.) Zopf. In some other Ina+ isolates, the threshold temperature fluctuated.

**Table 1. Ice nucleation activity (INA) of five *Fusarium* species, and ice nucleation threshold temperatures at four different ages**

| SRSF No.[a] | Host | Location | Repl. | INA[b] 7 d | INA[b] 14 d | INA[b] 21 d | INA[b] 28 d | Threshold temperatures[c] (°C) 7 d | Threshold temperatures[c] (°C) 14 d | Threshold temperatures[c] (°C) 21 d | Threshold temperatures[c] (°C) 28 d |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Fusarium acuminatum* (control) | | | | | | | | | | | |
| 116 | *Medicago sativa* L. | Pennsylvania, USA | A | + | + | + | + | -5.1 | -3.8 | -3.8 | -3.5 |
| | | | B | + | + | + | + | -4.6 | -3.9 | -3.6 | -4.4 |
| 368 | *Pisum sativum* L. | Quebec, Canada | A | + | + | + | + | -4.8 | -4.1 | -4.3 | -4.5 |
| | | | B | + | + | + | + | -4.6 | -3.9 | -3.5 | -4.3 |
| *Fusarium avenaceum* (control) | | | | | | | | | | | |
| 250 | *Medicago sativa* L. | Quebec, Canada | A | + | + | + | + | -5.3 | -3.8 | -3.7 | -3.9 |
| | | | B | + | + | + | + | -4.3 | -3.9 | -4.2 | -4.0 |
| 411 | *Medicago sativa* L. | Quebec, Canada | A | + | + | + | + | -3.8 | -3.5 | -3.6 | -3.4 |
| | | | B | + | + | + | + | -3.8 | -2.9 | -3.6 | -4.0 |
| *Fusarium oxysporum* | | | | | | | | | | | |
| 39 | Unknown | Manitoba, Canada | A | - | - | - | - | -9.2 | —[d] | — | — |
| | | | B | - | - | - | - | — | — | — | -9.4 |
| 41 | *Trifolium pratense* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | -8.1 | — | — |
| 114 | *Medicago sativa* L. | Pennsylvania, USA | A | - | - | - | - | -9.8 | — | — | — |
| | | | B | - | - | - | - | -9.7 | — | — | — |
| 115 | *Medicago sativa* L. | Maryland, USA | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | -8.9 |
| 117 | *Medicago sativa* L. | Maryland, USA | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | -9.8 |
| 120 | *Medicago sativa* L. | Maryland, USA | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | -7.5 | — |
| 242 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | -7.5 | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |

**Table 1. Ice nucleation activity (INA) of five *Fusarium* species, and ice nucleation threshold temperatures at four different ages (suite)**

| SRSF | | | | INA[b] | | | | Threshold temperatures[c] (°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No.[a] | Host | Location | Repl. | 7 d | 14 d | 21 d | 28 d | 7 d | 14 d | 21 d | 28 d |
| 245 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | -5.5 | — |
| 248 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | -9.4 | — |
| 249 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | -9.3 | -6.7 | -8.5 |
| | | | B | - | - | - | - | — | -9.1 | -5.9 | -8.9 |
| 252 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 260 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | -5.7 | — | — | -9.2 |
| | | | B | - | - | - | - | — | — | — | — |
| 296 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 300 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 301 | *Vicia faba* L. | Quebec, Canada | A | - | + | + | + | — | -3.0 | -2.8 | -2.5 |
| | | | B | - | + | + | + | -9.8 | -2.8 | -3.7 | -1.0 |
| 304 | *Solanum tuberosum* L. | Quebec, Canada | A | - | - | - | - | -7.4 | — | -5.2 | — |
| | | | B | - | - | - | - | — | — | — | — |
| 314 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 322 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | -5.6 | — |
| | | | B | - | - | - | - | — | -7.9 | — | — |
| 334 | *Medicago sativa* L. | Quebec, Canada | A | + | - | - | - | — | — | — | — |
| | | | B | - | - | + | - | — | — | — | — |
| 336 | *Phaseolus vulgaris* L. | Quebec, Canada | A | - | - | + | - | — | — | -6.7 | — |
| | | | B | - | - | + | - | -9.6 | -9.2 | -5.7 | -8.3 |

| SRSF No.[a] | Host | Location | Repl. | INA[b] | | | | Threshold temperatures[c] (°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 7 d | 14 d | 21 d | 28 d | 7 d | 14 d | 21 d | 28 d |
| 337 | *Phaseolus vulgaris* L. | Alberta, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 338 | *Phaseolus vulgaris* L. | Alberta, Canada | A | - | - | + | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 340 | *Pisum sativum* L. | Alberta, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 352 | *Pisum sativum* L. | Quebec, Canada | A | - | - | - | - | -8.3 | — | — | — |
| | | | B | - | - | - | + | — | — | -3.6 | -5.7 |
| 359 | *Pisum sativum* L. | Quebec, Canada | A | - | - | - | - | -9.1 | — | -7.9 | — |
| | | | B | - | - | - | - | -7.5 | -6.4 | — | — |
| 360 | *Pisum sativum* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | -10.0 | — | — |
| 376 | *Phaseolus vulgaris* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 398 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | + | — | — | — | -9.0 |
| 527 | *Lupinus albus* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 528 | *Lupinus albus* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | -7.4 | -10.0 | — |
| 529 | *Lupinus albus* L. | Quebec, Canada | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 573 | *Mangifera indica* L. | Malaysia | A | - | - | - | - | — | — | — | -8.3 |
| | | | B | - | - | - | - | -9.4 | — | — | — |
| 574 | Insects | Malaysia | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | -10.0 | — |
| 575 | *Cucumis melo* L. | Malaysia | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |

**Table 1. Ice nucleation activity (INA) of five *Fusarium* species, and ice nucleation threshold temperatures at four different ages (suite)**

| SRSF No.[a] | Host | Location | Repl. | INA[b] | | | | Threshold temperatures[c] (°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 7 d | 14 d | 21 d | 28 d | 7 d | 14 d | 21 d | 28 d |
| 576 | *Asparagus officinalis* L. | Malaysia | A | - | + | + | - | — | -3.6 | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 596 | Lab. contamination | Quebec, Canada | A | - | - | - | + | -5.5 | — | -9.7 | -7.2 |
| | | | B | - | - | - | - | — | — | — | — |
| 676 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 708 | *Medicago sativa* L. | Quebec, Canada | A | - | - | - | + | — | — | — | — |
| | | | B | - | - | - | - | — | -9.8 | — | — |
| 709 | *Medicago sativa* L. | Quebec, Canada | A | - | + | + | + | -6.0 | -1.8 | -3.8 | -1.0 |
| | | | B | + | + | + | + | -3.3 | -1.4 | -1.5 | -1.0 |
| 710 | Lab. contamination | Quebec, Canada | A | + | + | + | + | — | -3.6 | -2.5 | -1.2 |
| | | | B | - | + | - | + | — | -4.0 | — | -2.0 |
| 711 | *Medicago sativa* L. | Ontario, Canada | A | - | - | - | - | — | -7.1 | — | — |
| | | | B | - | - | - | - | — | — | — | -9.9 |
| 712 | *Medicago sativa* L. | Ontario, Canada | A | - | - | + | - | — | -4.0 | — | — |
| | | | B | - | - | + | + | — | — | -3.7 | -2.6 |
| *Fusarium sporotrichioides* | | | | | | | | | | | |
| 538 | *Zea mais* L. | Ontario, Canada | A | - | - | - | - | — | -6.7 | -7.8 | — |
| | | | B | + | - | - | - | -8.2 | — | — | — |
| 607 | Hay | Saskatchewan, Canada | A | - | - | - | - | -9.6 | — | — | — |
| | | | B | - | - | - | - | — | — | -9.3 | — |
| 608 | *Saccharum officinarum* L. | Guyana | A | - | - | - | - | -7.4 | — | — | -- |
| | | | B | - | - | - | - | — | — | -8.9 | -6.1 |
| 609 | Turf | Alberta, Canada | A | - | - | - | - | -9.3 | -9.7 | — | — |
| | | | B | - | - | + | - | — | — | — | — |

| SRSF No.[a] | Host | Location | Repl. | INA[b] 7 d | 14 d | 21 d | 28 d | Threshold temperatures[c] (°C) 7 d | 14 d | 21 d | 28 d |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 679 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | -9.7 | — | — | — |
| 692 | Unknown | Unknown | A | - | - | - | - | — | — | -7.0 | — |
| | | | B | - | - | - | - | -9.4 | — | — | — |
| 693 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 694 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 695 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | -9.5 | — | — | — |
| 696 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | -7.7 | — | — | — |
| 697 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 698 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | -7.8 | -9.1 | — |
| 699 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | -7.4 | — | — | — |
| 700 | Unknown | Unknown | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| *Fusarium tricinctum* | | | | | | | | | | | |
| 121 | *Medicago sativa* L. | Pennsylvania, USA | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | -8.8 | -9.8 | — |
| 612 | *Brassica napus* L. | Alberta, Canada | A | + | + | + | + | -3.9 | -3.6 | -3.7 | -3.5 |
| | | | B | + | + | + | + | -4.0 | -3.9 | -2.6 | -3.9 |

**Table 1. Ice nucleation activity (INA) of five *Fusarium* species, and ice nucleation threshold temperatures at four different ages (suite)**

| SRSF No.[a] | Host | Location | Repl. | INA[b] 7 d | INA[b] 14 d | INA[b] 21 d | INA[b] 28 d | Threshold temperatures[c] (°C) 7 d | Threshold temperatures[c] (°C) 14 d | Threshold temperatures[c] (°C) 21 d | Threshold temperatures[c] (°C) 28 d |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 663 | *Hordeum vulgare* L. | Poland | A | - | + | - | - | — | -5.5 | -9.5 | -9.0 |
| | | | B | - | - | - | - | -7.4 | -8.3 | -8.2 | -9.2 |
| 664 | *Triticum aestivum* L. | Poland | A | - | - | - | - | — | — | — | — |
| | | | B | - | - | - | - | — | — | — | — |
| 665 | *Hordeum vulgare* L. | Poland | A | - | + | - | + | -6.4 | -5.7 | -5.6 | -4.8 |
| | | | B | + | + | + | + | -5.7 | -4.3 | -4.7 | -5.2 |
| 666 | Soil | Italy | A | + | - | + | + | -5.2 | -7.0 | -1.0 | -4.8 |
| | | | B | - | + | + | + | -6.8 | -6.3 | -5.2 | -4.9 |
| 684 | Unknown | Unknown | A | - | + | + | + | -6.1 | -4.5 | -4.4 | -4.4 |
| | | | B | + | + | + | + | -4.9 | -5.1 | -4.0 | -4.8 |
| 685 | Soil | Poland | A | + | + | + | + | -5.1 | -4.8 | -4.3 | -4.7 |
| | | | B | + | + | + | + | -4.7 | -4.5 | -4.3 | -4.0 |
| 686 | Unknown | Poland | A | + | + | + | + | -4.8 | -4.8 | -4.2 | -3.9 |
| | | | B | + | + | + | + | -4.3 | -5.8 | -4.3 | -4.4 |
| 687 | Soil | Poland | A | + | + | + | + | -6.8 | -5.0 | -4.9 | -5.0 |
| | | | B | + | + | + | + | -4.4 | -4.6 | -4.4 | -4.4 |
| 688 | Soil | Poland | A | - | - | - | - | — | -9.4 | -9.4 | — |
| | | | B | - | - | - | - | — | -9.7 | — | -9.4 |
| 689 | Soil | Poland | A | - | - | - | - | — | -6.2 | -7.2 | -6.4 |
| | | | B | - | - | + | - | -9.3 | -5.8 | -6.5 | -6.0 |
| 690 | Soil | Poland | A | - | - | - | - | — | — | -9.6 | -9.8 |
| | | | B | - | - | - | - | -10.0 | — | — | -9.7 |
| 691 | Soil | Poland | A | - | - | - | - | — | -9.7 | -9.3 | -9.6 |
| | | | B | - | - | - | - | -8.8 | -8.5 | -9.8 | -9.4 |

[a] Collection from Station de recherches de Sainte-Foy (now Soils and Crops Research and Development Centre).
[b] At -5°C as detected by the rapid tube nucleation test : + indicates freezing within 10 min ($Ina^+$); - indicates no freezing ($Ina^-$).
[c] Temperature at which crystallization was triggered when the temperature of the tube contents was lowered from 0°C to -10°C at a rate of 0.1°C min $^{-1}$.
[d] — means that no freezing had crystallization occurred when the temperature reached -10°C.

The present finding of ice-nucleating activity at warm temperatures in another species of *Fusarium* suggests the need to screen even more fungal isolates for ice nucleation, including species other than *Fusarium*, and suggests that ice nucleation activity plays an important role in either virulence or environmental compatibility and survival. The role of ice nucleation in fungi needs to be investigated further.

The method used here to detect INA was simple and efficient. It allows to determine quickly if a given culture is Ina$^+$ or not. However, as opposed to the drop-freezing assay (Vali 1995), the rapid tube nucleation test can neither be used to quantify ice nuclei, nor to establish the cumulative ice nucleation spectrum. Pouleur *et al.* (1992) suggested the use of ice nucleation activity as an aid for the identification of *Fusarium* species. We know now that four fungal species are able to produce ice nuclei: *F. acuminatum, F. avenaceum, F. oxysporum* and *F. tricinctum*. This characteristic may prove useful in taxonomic treatment of these species.

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