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# Effects of fertilization with ammonium sulphate and potassium sulphate on the development of Sphaeropsis sapinea in Corsican pine

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**Abstract**

Potted plants of *Pinus nigra* subsp. *laricio* were fertilized with ammonium sulphate (AS) and potassium sulphate (PS) each year during 3 consecutive years. The then 5-year-old plants were artificially inoculated with *Sphaeropsis sapinea*. The fungus caused much bigger bark necroses in plants that had been fertilized with AS, than in plants fertilized with PS. It is concluded that *P. nigra* growing in poor sandy soil becomes more susceptible to *S. sapinea* when fertilized with an excess of AS. This may explain partly the increased incidence of *S. sapinea* in *P. nigra* stands in areas with a high rate of atmospheric $NH_4$-deposition in the Netherlands.

*Additional keywords: Diplodia pinea*, nitrogen deposition, air pollution, plant water potential, *Pinus nigra*, predisposition.

## Introduction

The pathogenic fungus *Sphaeropsis sapinea* (Fr.) Dyko & Sutton occurs throughout the temperate regions of the world. It causes shoot blight, bark necroses, crown wilt and blue stain in conifers, mainly in pine species, especially those growing under environmental stress (Punithalingam and Waterston, 1970). From 1982 to 1985 there was an epidemic of *S. sapinea* shoot blight and bark necrosis in *Pinus nigra* and *P. sylvestris* in the Netherlands (Kam and Van Dam, 1987), causing entire stands to decline in conditions otherwise suitable for pine growth. Symptoms caused by *S. sapinea* had never previously been recorded in the Netherlands even though the fungus had been present for at least a century (Hazenberg, 1866). Previous research indicated that the epidemic was most serious in areas with high air-borne nitrogen deposition: analysis of needles from stands seriously or slightly attacked by *S. sapinea* showed that in *P. nigra* and *P. sylvestris* the disease incidence correlated positively with foliar nitrogen content and with subsequent potassium deficiency (Ronse et al. 1986; Roelofs et al., 1985; Boxman and Van Dijk, 1988). It seems that the development of fungal diseases is generally favoured by an excess of N and a deficiency of K (Huber, 1980).

Most of the nitrogen deposition in the epidemic area is ammonium-N, mainly originating from animal slurry applied on adjacent farmland. The ammonium inputs in these forest soils have been estimated to be 40 - 70 kg $ha^{-1}$ $yr^{-1}$ (average values) to 120 - 270 kg $ha^{-1}$ $yr^{-1}$ of pure nitrogen near farms (Boxman and Van Dijk, 1988).

Most of the nitrogen is deposited as ammonium sulphate formed by interaction of ammonia ($NH_3$) with sulphur dioxide (Breemen et al., 1982).

In this paper we present experimental corroboration of the field observations that ammonium input may favour the development of an epidemic of *S. sapinea*.

## Material and methods

To study the effects of deposition of ammonium sulphate ($(NH_4)_2SO_4$, abbreviated AS) and of potassium sulphate ($K_2SO_4$, abbreviated: PS) on the development of *S. sapinea* a fertilization/inoculation experiment was carried out in the greenhouse.

*Plant material and soil.* In spring 1986 300 two-year-old plants of *Pinus nigra* subsp. *laricio* (Corsican pine) were planted in 60 containers holding 12 kg of a homogenized dry humus podzol developed in cover sand (5 plants per container). This substrate was taken from the upper mineral horizon (0-25 cm) near Epe, (Central Netherlands) which is not subject to excessive atmospheric N-deposition. The effect of atmospheric N-deposition on the soil N status is reflected in the $NH_4^+$ and $NO_3^-$ concentration of this Epe soil when compared to an identical soil near Venray (Southern Netherlands), which is subject to a high rate of N deposition (Table 1). The plants were watered with demineralized water; to prevent leaching of nutrients the watering matched transpiration and a saucer was placed under each pot. In spring 1987 the number of plants were adjusted to 3 per container unless otherwise indicated.

Table 1. Soil chemical data of the substrate used in the experiment (Epe) and in an area with a high rate of atmospheric N-deposition (Venray) in mg N $kg^{-1}$

| | N | | | | pH-KCl | Organic matter (%) | Total P (mg P/100 g) |
|---|---|---|---|---|---|---|---|
| | total | mineral | $NH_4^+$ | $NO_3^-$ | | | |
| Venray | 820 | 36.9 | 32.0 | 4.0 | 3.0 | 5.9 | 7 |
| Epe | 740 | 9.4 | 9.4 | 0.2 | 3.4 | 3.6 | 5 |

*Fertilization.* At the start of the experiment the phosphorus availability in the containers was optimized by a single overall fertilization of 3.6 g of calcium monophosphate [$Ca(H_2PO_4)_2.H_2O$] in each container, equivalent to 84 mg P per kg of soil. The 2-year-old plants were then split into subgroups, each of which received one of 5 levels of AS annually during 2 consecutive years. Within each N treatment half of the containers were additionally treated with PS. In the third year an overall gift of nitrogen (in the form of AS) was given to prevent N deficiency in the unfertilized plants and to maintain the various levels of N contents of the plants in the different treatments. Amounts of fertilizer were based on the pot surface and expressed as kg of the elements $ha^{-1}$. The fertilization scheme is given in Table 2. The total annual fertilization was given in 4 applications in a two-month period during flushing and shoot elongation. Most of the plants in treatments 5 and 10 (Table 2) died in the course of the

Table 2. Fertilization scheme; N given as ammonium sulphate and K given as potassium sulphate. Data given as equivalents of kg N and K·ha$^{-1}$.

| Treatment | 1986 | | 1987 | | 1988 | |
|---|---|---|---|---|---|---|
| | June | July | April | May | April | May |
| | N | K | N | K | N | K |
| 1 | 0 | 0 | 0 | 0 | 100 | 0 |
| 2 | 75 | 0 | 37.5 | 0 | 100 | 0 |
| 3 | 150 | 0 | 75 | 0 | 100 | 0 |
| 4 | 300 | 0 | 150 | 0 | 100 | 0 |
| 5 | 600 | 0 | 300 | 0 | 100 | 0 |
| 6 | 0 | 100 | 0 | 50 | 100 | 0 |
| 7 | 75 | 100 | 37.5 | 50 | 100 | 0 |
| 8 | 150 | 100 | 75 | 50 | 100 | 0 |
| 9 | 300 | 100 | 150 | 50 | 100 | 0 |
| 10 | 600 | 100 | 300 | 50 | 100 | 0 |

experiment, apparently because of overfertilization, and the data from those treatments have therefore been omitted in the subsequent tables. The containers were placed in 2 blocks, each block containing the 10 fertilization treatments randomly divided. The number of plants available at the time of inoculation are given in Table 3.

*Needle analyses.* Three days before inoculation with *S. sapinea* the chemical composition of the needles was analysed using samples of 4 needles per needle age class of each plant, pooled within treatment and age class and subdivided into 2 subsamples. The N content was measured with a Technicon AAII system, the K content was determined with a Technicon flame photometer IV; the other elements were measured with an Inductively Coupled Plasma spectrometer (IL Plasma 200).

Table 3. Number of plants inoculated in the various fertilization treatments (treatment numbers refer to Table 2)

| Fertilization treatment | Block 1 | | Block 2 | |
|---|---|---|---|---|
| | inoculated | control | inoculated | control |
| 1 | 6 | 3 | 6 | 3 |
| 2 | 6 | 3 | 6 | 3 |
| 3 | 6 | 3 | 6 | 3 |
| 4 | 3 | 1 | 2 | 1 |
| 6 | 6 | 3 | 6 | 3 |
| 7 | 6 | 3 | 6 | 3 |
| 8 | 6 | 3 | 6 | 3 |
| 9 | 5 | 2 | 5 | 2 |

*Water potential.* As *Sphaeropsis* epidemics have been related to drought stress (Chou, 1987), the water potential of 3 randomly chosen individual pairs of the previous year's needles of 3 plants was measured using the pressure chamber technique (Scholander et al. 1965; Johnson and Nielsen, 1969). Prior to measuring the water potential the soil in the containers was kept at field capacity for 2 days so that the needle water potential would not be influenced by differences in soil water content.

*Inoculation.* In August of the third vegetation period (1988) 87 of the by then 5-year-old plants were inoculated with mycelium of a pure culture of *S. sapinea* growing on malt agar. The isolate (Dsk 747-5) was obtained from a pycnidium of the fungus on *P. sylvestris*. From the plants to be inoculated a disk of bark was removed from the stems on the 1986 internodium using a 5 mm cork borer, and a 5 mm disc of maltagar was placed in the well with the mycelium on the cambium. In 42 control plants, agar without mycelium was put onto the cambium. The inoculation sites were kept moist for 48 hours by covering them with wet cotton wool sealed with parafilm.

*Assessment.* The growth of *Sphaeropsis* in the bark was assessed by measuring the maximum length of the bark necroses which developed as a result of the inoculation. The assessment method was destructive because it involved removing the outer bark. As the rate of growth of the fungus could not be predicted fungal development was assessed 15, 20 and 25 days after inoculation. On each date a randomly chosen one-third of the plants was assessed (Table 6).

*Statistics.* The data collected on the chemical composition of the needles, on the needle water potential and on the length of the necroses were analysed statistically for significant differences using the Genstat 5 programme ANOVA as a mixed model.

**Results**

*Effects of the fertilization on the chemical composition of the needles.* Table 6 shows that the fertilization with AS had resulted in clear differences of N content in the needles of the various treatments ($P < 0.001$). In the plants fertilized with AS+PS the N content was always less than in the plants fertilized with AS alone ($P < 0.001$). In the plants not fertilized with PS, symptoms of K deficiency were visible (pale yellow needle tips); the foliar concentration of K was low and the N : K ratio was 100 : 13-28, which is unfavourable because in Corsican pine with N = 100, K should be above 25 (Van den Burg, 1988). In the plants fertilized with PS the N : K ratio was 100 : 53-67, which is optimal for Corsican pine. The phosphorus availability was sufficient in all treatments. As a result of the fertilization with AS the calcium content dropped alarmingly to values which are known to cause Ca-deficiency (Van den Burg, 1988). The concentrations of magnesium, manganese, zinc and aluminium generally decreased with increasing AS gifts.

*Effects of the fertilization on the needle water potential.* Table 5 and Figure 1 show that in the treatments 3, 4, 8 and 9 the needle water potential was more negative than in treatments 1, 2, 6 and 7. Thus, large AS gifts reduced the water potential. Fertilization with PS did not affect the water potential significantly.

Table 4. Mineral content of current and one-year-old needles on 8-8-1988 (3 days before inoculation) and mineral ratios. N, K, Ca, Mg & P in ‰ dw; Mn, Zn & Al in mg/kg dw.

| *1987 needles* | | | | | | | | | N = 100 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment* | N | P | K | Ca | Mg | Mn | Zn | Al | P | K | Mg |
| 1 | 1.31 | 0.12 | 0.11 | 0.48 | 0.11 | 390 | 91 | 676 | 9 | 8 | 9 |
| 2 | 1.63 | 0.14 | 0.18 | 0.32 | 0.10 | 407 | 36 | 440 | 9 | 11 | 6 |
| 3 | 1.84 | 0.13 | 0.15 | 0.19 | 0.09 | 286 | 51 | 237 | 7 | 8 | 5 |
| 4 | 2.06 | 0.16 | 0.28 | 0.16 | 0.09 | 262 | 18 | 128 | 8 | 14 | 4 |
| 6 | 1.07 | 0.09 | 0.54 | 0.45 | 0.08 | 472 | 83 | 569 | 8 | 50 | 7 |
| 7 | 1.16 | 0.09 | 0.68 | 0.35 | 0.07 | 403 | 67 | 457 | 8 | 59 | 6 |
| 8 | 1.29 | 0.09 | 0.64 | 0.22 | 0.06 | 312 | 43 | 288 | 7 | 50 | 4 |
| 9 | 1.64 | 0.11 | 0.56 | 0.15 | 0.06 | 252 | 33 | 164 | 7 | 34 | 4 |
| *1988 needles* | | | | | | | | | N = 100 | | |
| Treatment* | N | P | K | Ca | Mg | Mn | Zn | Al | P | K | Mg |
| 1 | 1.57 | 0.20 | 0.31 | 0.16 | 0.09 | 151 | 34 | 197 | 12 | 20 | 6 |
| 2 | 1.94 | 0.19 | 0.25 | 0.08 | 0.08 | 109 | 19 | 101 | 10 | 13 | 4 |
| 3 | 1.97 | 0.19 | 0.39 | 0.02 | 0.08 | 76 | 9 | 47 | 9 | 20 | 4 |
| 4 | 1.98 | 0.17 | 0.56 | 0.06 | 0.08 | 125 | 27 | 31 | 9 | 28 | 4 |
| 6 | 1.32 | 0.12 | 0.89 | 0.14 | 0.07 | 147 | 24 | 228 | 9 | 67 | 5 |
| 7 | 1.17 | 0.12 | 0.80 | 0.05 | 0.05 | 95 | 13 | 153 | 10 | 68 | 4 |
| 8 | 1.32 | 0.13 | 0.88 | 0.06 | 0.05 | 80 | 15 | 110 | 10 | 67 | 4 |
| 9 | 1.52 | 0.14 | 0.89 | 0.02 | 0.05 | 55 | 11 | 30 | 9 | 59 | 3 |

* Treatment numbers refer to Table 2.

*Effects of the fertilization on the growth of S. sapinea in the bark.* The length of the necroses that developed as a result of the infection varied considerably between the plants of one fertilization level (Table 6). As a result, the length of the necroses measured at 15, 20 and 25 days after infection did not differ significantly as far as period after inoculation is concerned. To calculate the overall effect of AS and PS fertilizations on the length of the necroses the data collected on the three assessment dates were therefore pooled. The statistical analyses resulted in the conclusion that the necroses on the plants fertilized with 150 and 300 kg N in the first year were longer than those fertilized with 0 and 75 kg N in that year ($P < 0.019$ on log scale). Further, *S. sapinea* caused much smaller necroses in the plants fertilized with AS+PS than in those fertilized with AS alone ($P < 0.002$ on log scale). The data of Table 6 are summarized in Figure 2. In the control plants a necrotic rim not exceeding 2 mm in width was visible at the edge of the wounds. It was considered to be a wound reaction; this was confirmed by reisolation.

Table 5. Water potential in MPa (negative) on 10 August 1988 (1 day before inoculation) from 06.00h to 22.00h (average of 3 previous year's needle fascicles from 3 plants).

| Treatment[1] | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | Sign[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.42 | 0.45 | 0.48 | 0.51 | 0.62 | 0.96 | 0.71 | 0.60 | 0.56 | a |
| 2 | 0.48 | 0.46 | 0.53 | 0.65 | 0.68 | 1.01 | 0.93 | 0.68 | 0.63 | a |
| 3 | 0.93 | 0.93 | 0.88 | 1.08 | 1.12 | 1.63 | 1.09 | 0.99 | 1.06 | b |
| 4 | 0.74 | 0.83 | 0.82 | 1.09 | 0.95 | 1.32 | 1.21 | 1.12 | 0.93 | b |
| 6 | 0.43 | 0.43 | 0.47 | 0.53 | 0.64 | 0.93 | 0.76 | 0.59 | 0.48 | a |
| 7 | 0.43 | 0.44 | 0.41 | 0.58 | 0.68 | 0.87 | 0.93 | 0.62 | 0.56 | a |
| 8 | 0.69 | 0.66 | 0.73 | 0.80 | 0.83 | 1.19 | 0.94 | 0.73 | 0.74 | b |
| 9 | 1.17 | 1.03 | 1.30 | 1.33 | 1.16 | 1.58 | 1.47 | 1.12 | 1.25 | b |

[1] Treatment numbers refer to Table 2.
[2] Data marked with different characters differ significantly ($P < 0.001$).

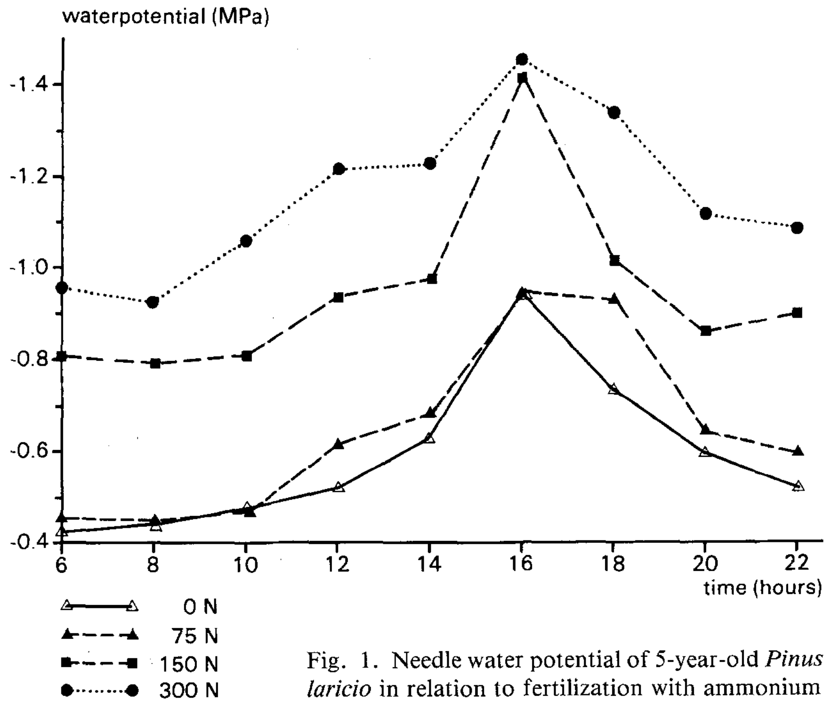


Fig. 1. Needle water potential of 5-year-old *Pinus nigra* subsp. *laricio* in relation to fertilization with ammonium sulphate.

## Discussion

It has been shown (Chou, 1978) that *S. sapinea* may attack healthy, not yet lignified current year's shoots of *Pinus radiata*. However, under normal physiological conditions the bark of the woody stem of *P. radiata* is resistant to attack by *S. sapinea*,

Table 6. Length of the barknecroses (mm), given in ascending order within treatment. Each number represents 1 necrosis on 1 plant. Treatment numbers refer to Table 2.

| Treatment | Necroses | | | | Average | Sign[2] | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | N | K |
| *15 days after inoculation* | | | | | | | |
| 1 | 16 | 37 | 67 | 130 | 63 | a | a |
| 2 | 17 | 25 | 25 | 60 | 32 | a | a |
| 3 | 15 | 68 | 70 | 135 | 72 | b | b |
| 4 | 38 | 50 | –[1] | –[1] | 44 | b | b |
| 6 | 12 | 13 | 16 | 17 | 15 | a | a |
| 7 | 12 | 13 | 15 | 17 | 14 | a | a |
| 8 | 10 | 14 | 22 | 34 | 20 | b | b |
| 9 | 15 | 22 | 28 | 60 | 31 | b | b |
| *20 days after inoculation* | | | | | | | |
| 1 | 19 | 19 | 22 | 115 | 44 | a | a |
| 2 | 30 | 54 | 54 | 120 | 65 | a | a |
| 3 | 95 | 106 | 115 | 117 | 108 | b | b |
| 4 | 55 | 99 | –[1] | –[1] | 77 | b | b |
| 6 | 10 | 14 | 18 | 21 | 16 | a | a |
| 7 | 12 | 12 | 18 | 35 | 22 | a | a |
| 8 | 17 | 24 | 29 | 65 | 34 | b | b |
| 9 | 20 | 46 | 112 | 115 | 73 | b | b |
| *25 days after inoculation* | | | | | | | |
| 1 | 15 | 20 | 21 | 33 | 22 | a | a |
| 2 | 15 | 19 | 30 | 70 | 34 | a | a |
| 3 | 59 | 114 | 126 | 220 | 130 | b | b |
| 4 | 155 | –[1] | –[1] | –[1] | 155 | b | b |
| 6 | 16 | 22 | 23 | 27 | 22 | a | a |
| 7 | 13 | 21 | 23 | 23 | 23 | a | a |
| 8 | 15 | 24 | 26 | 42 | 27 | b | b |
| 9 | 43 | 170 | –[1] | –[1] | 107 | b | b |

[1] Plant was already dead at the time of infection.
[2] Data marked with different characters differ significantly on log scale (for N: $P < 0.019$; for K: $P < 0.001$).

and this resistance may be broken under stress conditions (Chou, 1987). These stress conditions are still ill-defined. In the experiment described here *S. sapinea* developed bigger necroses in the bark of the woody stem of *P. nigra* when the plants were fertilized with large amounts of AS, suggesting that the plants were predisposed to attack by *S. sapinea* as a result of the large AS gifts. Large AS gifts disrupted the mineral balance in the needles: with increasing ammonium levels the N concentration in the needles increased, but the concentrations of calcium, magnesium, manganese, zinc

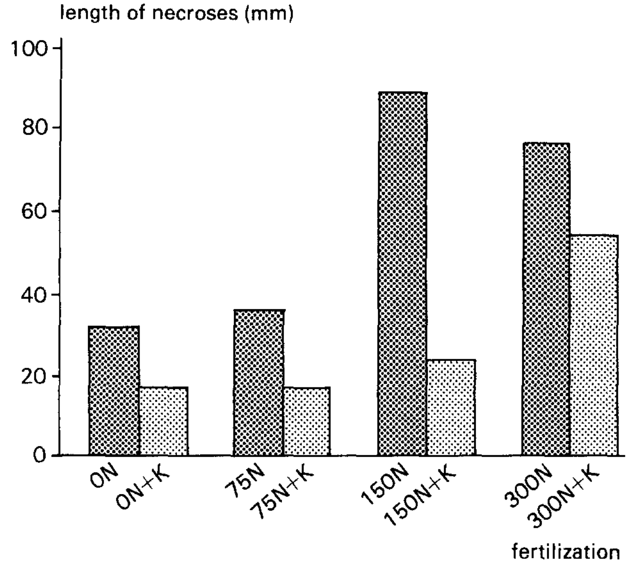


Fig. 2. The effect of fertilization with nitrogen (N) and with nitrogen plus potassium (N + K) on the development of bark necroses caused by *S. sapinea* in Corsican pine after artificial inoculation.

and aluminium generally decreased. This decrease could not be attributed to the nutrients being 'diluted' by the faster growth of the plants, because in this experiment the growth of the plants decreased with increasing ammonium gifts (De Kam et al., 1990). It was expected that as a consequence of the $K^+$-$NH_4^+$ antagonism in the soil the K concentration in the needles would also decrease with increasing ammonium gifts. But Table 4 shows that the K-concentration increased with increasing ammonium gifts, whereas the N : K ratios were only affected in the plants fertilized with AS + PS, i.e. in the plants with sufficient potassium. This seeming contradiction might be explained by the fact that in our experiment the leaching out of nutrients was prevented (which is not the case in the field), so that the $K^+$ ions remained available for the plants.

Fertilization with PS caused much smaller necroses (Figure 2), suggesting that the tolerance of the plants to *S. sapinea* was partially restored when potassium was no longer deficient. This effect was not caused by the AS fertilization, but by the improvement of plant K status by PS fertilization (treatments 1-4 versus 6-9).

As shown in Table 5, high levels of ammonium sulphate caused a significant decrease of the needle water potential, which reached values as small as minus 1.6 MPa. This decrease may have been caused by an increased shoot/root ratio, as it has been shown that the formation of roots and the development of mycorrhizas is hampered by excessive ammonium depositions (Boxman and Van Dijk, 1988; Van Dijk et al., 1990). We did not study the effect of such low water potentials on *P. nigra* plants, but it is probable that the plants concerned suffered from water shortage and this may have increased their susceptibility to *Sphaeropsis*, as has been suggested by Chou (1987) and by Bachi and Peterson (1985). Although the underlying physiological mechanisms that reduce the tolerance of the older bark tissue are not yet understood, our results give empirical support to field observations that excessive deposition of ammonium on dry sandy soils in which potassium availability is barely adequate may predispose the bark tissue of woody stems of Corsican pine to an attack of *S. sapinea*.

As several plant diseases have been shown to be stimulated by heavy nitrogen fertilization (Huber, 1980), it may be surmised that increasing air borne nitrogen deposition may encourage the occurrence of other plant pathogens too, especially on soils where nutrients and water are marginally available.


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