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First report of susceptibility of *Cannabis sativa* to *Nacobbus celatus*

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In the cultivation of *Cannabis sativa* for medicinal purposes, the harvested product consists of the female inflorescences. These contain the glandular trichomes where phytocannabinoids, flavonoids and terpenes of medicinal interest accumulate. In Argentina, after decades of prohibition of *C. sativa* cultivation, new regulations were enacted in 2017, initiating a gradual process of resurgence and new possibilities around this species. These changes generated demand for agronomic, pharmaceutical and medicinal information, and positioned scientific and technical institutions as key actors for knowledge development and the provision of reliable information. One of the open questions concerns which region will host the productive and value-added chain for cannabis.

The La Plata Horticultural Belt is located on the outskirts of the city of La Plata and constitutes the most important intensive agricultural production area in the country, supplying fresh vegetables to the Buenos Aires metropolitan area, where more than 13 million people live. This productive zone has 7100 ha of greenhouses (Ferraris & Ferrero, 2018), and its production units have extensive experience in the agronomic practices typical of intensive crop management, such as pruning, transplanting, manual harvesting and continuous monitoring of pests and diseases. These technical and productive characteristics position the region as a promising area for cannabis cultivation. However, for several decades the region has been affected by a high-impact pest, historically identified as *Nacobbus aberrans s.l.* (Nico, 2014) and recently described as the species *Nacobbus celatus* (Nematoda: Pratylenchidae) (Lax *et al*., 2021)

Nematodes of the genus *Nacobbus*, commonly known as false root-knot or rosary nematodes, cause significant yield losses in horticultural crops across the American continent. Broadly defined under the *Nacobbus aberrans* complex (*sensu lato*), their presence and devastating damage have been reported in the United States, Mexico, Ecuador, Peru, Bolivia, Chile and Argentina (Manzanilla-López *et al*., 2002). Due to this destructive potential, the genus is classified as a high-risk quarantine pest by the European Union, Israel and Brazil (EPPO, 2026), and was ranked among the top ten plant-parasitic nematode species of global economic importance (Jones *et al*., 2013). Within this genus, *Nacobbus celatus* has been recently described as a distinct species native to the lowlands of Argentina. Specifically, populations from the La

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Plata region (Colonia Urquiza) have been assigned to the 'sugar beet group' (sugar beet pathotype), characterised by their ability to infect sugar beet, tomato, and pepper, but not potato (Vovlas *et al*., 2007). In this region, *N. celatus* severely threatens horticultural sustainability; Doucet & Lax (2005) have documented that, in severe cases, infestations in solanaceous crops completely compromise crop survival.

At the cellular level, the damage varies according to the life stage of the parasite: while infective juveniles and males are migratory and cause cortical necrosis and cavities, only the mature sedentary females induce specialised feeding sites known as syncytia. These syncytia are functional structures derived from the partial fusion of adjacent cells, which cause the displacement of xylem and phloem and alter the normal flow of water and nutrients (Vovlas *et al*., 2007; Garita *et al*., 2021). The objective of this study was to evaluate the susceptibility or resistance of *Cannabis sativa* to attack by *N. celatus* and whether this attack affects cannabinoid production.

The experiment had a completely randomised design with two treatments and 15 replications per treatment. Each plant constituted one replication. The treatments were non-inoculated plants and plants inoculated with *N. celatus*. Data were analysed by one-way analysis of variance (ANOVA), and means were compared using Fisher's LSD test with the statistical program InfoStat.

Cuttings were taken from mother plants of *Cannabis sativa* 'Ballena franca' and rooted in a substrate composed of soil, perlite and vermiculite in a 1:1:1 ratio. After 21 days, 30 seedlings were transplanted into 10 l pots containing topsoil from an Argiudol previously tyndallised (three consecutive 1 h heating cycles at 100°C) to eliminate native nematodes and other soil-borne pathogenic microorganisms. The day after transplanting, half of the plants were inoculated with 5000 eggs of *N. celatus*, establishing the two treatments: non-inoculated control plants and inoculated plants. Nematode eggs used for inoculation were obtained from tomato plants (*Solanum lycopersicum*) grown in sterile substrate under controlled environmental chambers. These plants maintained a stock population of *N. celatus* originally collected from Colonia Urquiza (La Plata, Buenos Aires), a locality situated within the La Plata Horticultural Belt. This population was genetically characterised as belonging to the 'Argentina 2' group and the 'sugar beet' pathotype by Vovlas *et al*. (2007), and was subsequently established as the reference for the formal description of *N. celatus* Lax *et al*. (2021) using an integrative taxonomic approach. As a positive control to verify inoculum viability, 10 tomato plants (*Solanum lycopersicum* 'Platense') were grown from seed, inoculated, and managed under the same conditions as the cannabis plants.

Cannabis plants were maintained in growth rooms with artificial lighting and controlled temperature of 27°C. For 40 days, plants were kept under a 16-h photoperiod to maintain vegetative growth, after which an 11-h photoperiod was applied for 50 days to stimulate flowering. The variables analysed were:

i) Flower Yield: Expressed as dry flower weight per plant (g plant^{-1}). All inflorescences from each plant were harvested and dried to determine total yield;
ii) Root Fresh Weight: Measured immediately after harvest (g plant^{-1});
iii) Cannabinoid Content: Expressed as mg per g of dry flower (mg g^{-1}). To ensure sampling homogeneity across treatments, the main apical inflorescence of each plant was selected and ground. Individual cannabinoids were identified and quantified using UHPLC-MS/MS. Chromatographic analyses were performed on a Thermo Scientific Vanquish UHPLC system coupled to a Thermo Scientific TSQ Quantum Access Max mass spectrometer equipped with a heated electrospray ionization (HESI) source (Martins *et al*., 2023);
iv) Number of galls per plant: Determined by visual inspection of the entire root system;
v) Number of eggs extracted from roots: The entire root system was processed using a modified agitation-extraction technique based on Hussey & Barker (1973) and Coolen (1979). Briefly, roots were macerated in a commercial blender with a sodium hypochlorite (NaOCl) solution, followed by purification and concentration using a modified centrifugation-flotation technique (Coolen, 1979);
vi) Nematode reproduction factor: RF = final population/initial population. Where the initial population represents the number of inoculated eggs, and the final population is the total number of eggs extracted from the roots at harvest. According to the classification by Charchar *et al*. (2003), host cultivars are categorised as resistant when RF = 0, intermediate resistant when $0 < RF \leqslant 1$, and susceptible when $RF > 1$.

At harvest, the roots of the tomato plants (used as a highly susceptible reference host) were removed and the presence of galls was observed. This confirmed that the egg inoculum used was effective for infection.

Table 1. Cannabinoid profile of *Cannabis sativa* inflorescences, infected and non-infected with *Nacobbus celatus*.

	CBD (mg g^{-1})	THC (mg g^{-1})	CBN (mg g^{-1})	CBDA (mg g^{-1})	THCA (mg g^{-1})	CBG (mg g^{-1})	Total acidic cannabinoids (mg g^{-1})	Total neutral cannabinoids (mg g^{-1})	Total cannabinoids (mg g^{-1})
Non-inoculated with *N. celatus* (n = 15)	3.06 a	4.87 a	0.97 a	95.3 a	55.74 a	1.05 a	151.08 a	7.93 a	161.03 a
Inoculated with *N. celatus* (n = 15)	2.94 a	5.04 a	0 b	94.7 a	58.9 a	0.95 a	153.6 a	7.9 a	162.6 a

CBD = cannabidiol; THC = Δ 9-tetrahydrocannabinol; CBN = cannabinol; CBDA = cannabidiolic acid; THCA = tetrahydrocannabinolic acid; CBG = cannabigerol; mg g^{-1} = mg cannabinoids per g of inflorescence; n = the number of biological replications. Means with the same letter in a column are not significantly different according to Fisher's LSD test ($P > 0{\cdot}01$)

The mean root fresh weight of cannabis plants showed no significant differences between non-inoculated (16.9 g) and inoculated plants (17.8 g), according to Fisher's LSD test ($P > 0{\cdot}01$). Total absence of galling was observed in non-inoculated plants, whereas parasitised plants exhibited between 35 and 50 galls per plant.

Nematode egg extraction and quantification from roots yielded an average of 13 218 eggs per plant and the reproduction factor (RF) was 2.64. According to the classification proposed by Charchar *et al*. (2003), these values confirm that *C. sativa* is a susceptible host for this nematode species. The ratio of eggs to root fresh weight ranged from 466 to 1021 eggs g^{-1}, with a mean ± SD of 747 ± 215 eggs g^{-1}. Flower yield was significantly reduced by *N. celatus* parasitism, averaging 28.2 g in healthy plants and 21.4 g in infected plants ($P < 0{\cdot}01$).

Analysis of cannabinoids produced by the flowers showed no differences in the content of tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabigerol (CBG), a precursor to other cannabinoids. There were also no differences in the acidic or neutral forms of the molecules, nor in total cannabinoid content. However, all samples from plants not inoculated with the nematode contained cannabinol (CBN), whereas CBN was consistently absent in inoculated plants (Table 1).

Although the La Plata Horticultural Belt presents high potential for *C. sativa* production due to its technical capacity and proximity to urban centres, the presence of *N. celatus* may be a critical limiting factor. This finding is highly relevant for other American countries, such as Bolivia, Peru, Ecuador, Chile, Mexico and the USA, where various species of the *N. aberrans* complex (*e.g.*, *N. bolivianus* and *N. aberrans* s.s.) cause devastating economic losses in other horticultural crops. As cannabis cultivation expands across the continent, these results establish a critical precedent. It is imperative to monitor the different species within the *N. aberrans* complex, as their wide host range and adaptive capacity pose a potential threat that requires a precautionary approach in regions where the genus is endemic. Currently, the primary control tool for this pest is chemical soil treatment, a practice often incompatible with the safety standards required for medicinal plant production.

This study constitutes the first report of the susceptibility of *C. sativa* to *N. celatus*. Notably, in the recent review by Bernard *et al*. (2022) regarding nematodes attacking cannabis, this species is not mentioned. Our results show that the severity of the infection is high even in short cycles: a 90-day cycle was sufficient to induce the formation of functional syncytia and cause a 24.1% reduction in flower yield.

While there were no significant differences in cannabinoid synthesis per g of flowers, the reduction in total flower biomass resulted in a significant decrease in total cannabinoid production per plant: 3479.64 mg in parasitised plants compared to 4541.04 mg in non-parasitised plants. Furthermore, the consistent presence of cannabinol (CBN) only in the control plants is noteworthy. Since CBN is a degradation product resulting from the oxidation of THC rather than enzymatic synthesis, its absence in inoculated plants suggests that parasitism might alter the phenological timing or the rate of secondary metabolite maturation.

In conclusion, *C. sativa* is a susceptible host of the endoparasitic nematode *N. celatus*. Parasitism significantly compromises crop yield and alters the cannabinoid profile, highlighting the need for integrated pest management strategies tailored to medicinal cannabis production.

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