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Susceptibilidad varietal y efecto de la fertilización nitrogenada sobre la incidencia de antracnosis en frutos de arándanos causada por *Colletotrichum fioriniae* (Varietal susceptibility and effect of nitrogen fertilization on the incidence of anthracnose caused by *Colletotrichum fioriniae* on blueberry fruits)

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RESUMEN

El impulso de programas de recambio varietal y la reciente identificación de *Colletotrichum fioriniae* afectando frutos de arándano en Chile, plantean la necesidad de evaluar la susceptibilidad a la antracnosis de nuevos cultivares comerciales y esclarecer la relación entre la severidad de esta enfermedad y la fertilización mineral. El objetivo de esta investigación fue determinar la susceptibilidad varietal y el efecto de la fertilización nitrogenada sobre la incidencia de antracnosis en arándanos. Se evaluaron ocho cultivares de arándanos, los cuales fueron fertilizados con cuatro dosis de nitrógeno (0, 0.16, 0.32 y 0.48 g planta día^{-1}). En estado de fruto verde, las plantas fueron inoculadas con *C. fioriniae* RGM 3330, junto con un control sin patógeno. Los frutos maduros se recolectaron en la cosecha para evaluar la incidencia y severidad de la antracnosis. Además, se inocularon hojas *in vitro* con *C. fioriniae* y *Botrytis cinerea* para evaluar la capacidad de estos hongos de infectar tejidos vegetativos. Los resultados indican que las variedades de cosecha intermedia (‘Duke’, ‘Last Call’ y ‘Legacy’) y tardía (‘Ochlockonee’ y ‘Victoria’) fueron más susceptibles a la antracnosis y que las condiciones ambientales fueron cruciales para la infección de *C. fioriniae*. La fertilización nitrogenada no mostró un efecto significativo sobre la incidencia y severidad de antracnosis en frutos ni en tejidos vegetativos. En las hojas, la fertilización tuvo un efecto sobre el área necrótica causada por *B. cinerea*. Estos resultados entregan información relevante para el desarrollo de programas fitosanitarios en arándano para el manejo de *C. fioriniae*.

ABSTRACT

The promotion of varietal replacement programs and the recent identification of *Colletotrichum fioriniae* affecting blueberry fruits in Chile raise the need to evaluate the susceptibility to anthracnose of new commercial cultivars and clarify the relationship between the severity of this disease and mineral fertilization. The objective of this research was to determine varietal susceptibility and the effect of nitrogen fertilization on the incidence of anthracnose in blueberries. Eight blueberry cultivars were evaluated, which were fertilized with four doses of nitrogen (0, 0.16, 0.32 and 0.48 g plant day-1). At the green fruit stage, the plants were inoculated with *C. fioriniae* RGM 3330, along with a pathogen-free control. Ripe fruits were collected at harvest to evaluate the incidence and severity of anthracnose. Additionally, leaves were inoculated in vitro with *C. fioriniae* and *Botrytis cinerea* to evaluate the ability of these fungi to infect vegetative tissues. The results indicate that mid-crop

(‘Duke’, ‘Last Call’ and ‘Legacy’) and late-crop varieties (‘Ochlockonee’ and ‘Victoria’) were more susceptible to anthracnose and that environmental conditions were crucial for C. fioriniae infection. Nitrogen fertilization did not show a significant effect on the incidence and severity of anthracnose in fruits or vegetative tissues. In the leaves, fertilization had an effect on the necrotic area caused by *B. cinerea*. These results provide relevant information for the development of phytosanitary programs in blueberries for the management of *C. fioriniae*.

CAPITULO I.

El consumo de arándanos (*Vaccinum* spp.) ha incrementado notoriamente, lo que ha sido acompañado por un aumento en el área de cultivo de este frutal a nivel mundial (Fang et al., 2020; Rodriguez-Saona et al., 2019). En las últimas dos décadas, Chile fue el país del hemisferio sur que lideró las exportaciones de arándano fresco hacia Estados Unidos y Europa, alcanzando su récord de exportación durante la temporada 2020/21 con 117.640 ton (IQconsulting, https://www.iqonsulting.com/yb/). Sin embargo, actualmente el país ha tenido una reducción de un 20% de las exportaciones durante la temporada 2022/23, en comparación con el año anterior (ODEPA, 2023). En paralelo, el incremento de los precios de los fertilizantes a nivel mundial, asociado a la guerra entre Rusia y Ucrania ha repercutido fuertemente en los costos de producción (Hebebrand et al., 2023). En este escenario, el recambio de variedades y los sistemas intensivos de producción, resultan indispensables para mejorar los rendimientos y la competitividad de este frutal menor (Fang et al., 2020; Mazzoni et al., 2020). Por ello, existe un alto interés por evaluar la adaptación de nuevos cultivares de arándanos de producción temprana de fruta, comparando su potencial respecto a los cultivares tradicionalmente producidos en Chile (Muñoz-Vega et al., 2016; Muñoz et al., 2023).

Por otra parte, la reciente identificación de la especie de *Colletotrichum fioriniae* (Marcelino & S. Gouli) R.G. Shivas & Y.P. Tan. causando antracnosis en frutos de arándano en Chile (Castro et al., 2022), plantea un desafío relevante, especialmente considerando que los hongos de poscosecha pueden reducir hasta en un 20% la productividad de este berry (Bell et al., 2021). Especies de hongos del género *Colletotrichum* son el agente causal de la antracnosis, una de las enfermedades más importantes de poscosecha en arándano en los Estados Unidos (Bell et al., 2021; Dean et al., 2012). Los síntomas de antracnosis se observan en el campo antes de cosecha y durante el almacenamiento en poscosecha, causando zonas hundidas sobre la superficie de frutos maduros, donde se desarrollan acérvulos que producen masas de conidias de color naranjo y que son visibles sobre los frutos afectados (Wharton & Schilder, 2008). *C. fioriniae*, es una especie que pertenece al complejo *C. acutatum*, la que se caracteriza por causar infecciones a temperaturas más moderadas que otras especies de este género (Salotti et al., 2022). Esta característica y las variaciones medioambientales asociadas con el cambio climático, han causado un incremento en la tasa de antracnosis en áreas de clima templado (Lee, 2019; Morkeliūnė et al., 2021), lo implica un riesgo para la producción de arándanos en Chile y plantea la necesidad de conocer la epidemiología, evaluar la susceptibilidad varietal y buscar soluciones para el adecuado manejo y control de esta enfermedad. El control de este patógeno se basa principalmente en la aplicación de fungicidas antes y después de la cosecha, no obstante aislados de *C. gloeosporioides* han mostrado resistencia a los fungicidas Tiofanato-Metilo (benzimidazol), Piraclostrobina (QoI group) y Boscalid (DDHI group), lo cual ha sido favorecido por el limitado número de compuestos disponibles para el manejo de esta enfermedad (Ali et al., 2019). Una alternativa para manejar la antracnosis es el uso o desarrollo de variedades resistentes a *Colletotrichum* spp. (Dowling et al., 2020; Panth et al., 2020). Lamentablemente, no existe suficiente información científica respecto a variedades que sean resistentes, y que a su vez se caractericen por tener una producción de fruta temprana o de media estación (Miles & Hancock, 2022; Polashock et al., 2005). Estudios previos, han evaluado la susceptibilidad a antracnosis causada por *C. acutatum* en distintos genotipos de arándano, reportando como resistentes las variedades 'Elliot', 'Legacy', 'Brigitta Blue', y como susceptibles a 'Jersey', 'Blue crop', 'Duke' y 'Flicker', entre otras (Miles et al., 2011; Phillips et al., 2018; Polashock et al., 2005). Pero no hay reportes actuales sobre la susceptibilidad de nuevas variedades de arándanos a esta enfermedad, por lo que es indispensable limitar el uso de nuevas variedades comerciales susceptibles para evitar el desarrollo de epífitas que afecten la productividad del arándano (Phillips et al., 2018) y el uso excesivo de fungicidas para su manejo.

Por otra parte, la nutrición mineral puede reducir o incrementar el desarrollo de enfermedades causadas por diferentes patógenos (Gupta et al., 2017; Walters & Bingham, 2007). Aunque la severidad de antracnosis es afectada por el manejo de la fertilización, su relación no ha sido dilucidada (Sun et al., 2020). (Nam et al., 2006) señalan que concentraciones altas de N y K incrementan la severidad de antracnosis en frutilla, mientras otros reportes describen el efecto inverso, indicando que la severidad de los síntomas de antracnosis disminuyen al aumentar la dosis de N, en cultivos de berries como *Rubus glaucus* Benth (Bautista-Montealegre et al., 2019) o frutales como *Olea europea* (Rodrigues et al., 2019). Por lo tanto, frente a la necesidad de un recambio varietal que permita incrementar la productividad del arándano, y considerando que la selección de nuevas variedades resulta decisivo para mejorar la competitividad de este cultivo en Chile, esta investigación buscó determinar la susceptibilidad de cultivares de arándanos nuevos y tradicionales a la antracnosis, causada por la especie recientemente reportadas en Chile *C. fioriniae*, y evaluar el efecto que tienen dosis crecientes de fertilización nitrogenada sobre la severidad de esta enfermedad en hojas y en frutos en poscosecha.

HIPÓTESIS

La incidencia de antracnosis en frutos de post cosecha de arándano, es afectada por la fertilización nitrogenada y la susceptibilidad varietal.

OBJETIVO GENERAL

Determinar la susceptibilidad varietal y el efecto de la fertilización nitrogenada sobre la incidencia de antracnosis en frutos de post cosecha de arándano.

OBJETIVOS ESPECÍFICOS

Evaluar el efecto de la aplicación de dosis crecientes de fertilización nitrogenada sobre la incidencia de antracnosis en frutos de post cosecha de arándano causada por *Colletotrichum fioriniae*.

Comparar la susceptibilidad de seis variedades de arándanos a antracnosis, en relación a una variedad resistente y una susceptible.

Evaluar la susceptibilidad de hojas a la inoculación *in vitro* de *Colletotrichum fioriniae* de plantas de arándanos de distintos cultivares y fertilizadas con dosis crecientes de nitrógeno.

REFERENCIAS

Abro, M. A., Lecompte, F., Bryone, F., & Nicot, P. C. 2013. Nitrogen fertilization of the host plant influences production and pathogenicity of *Botrytis cinerea* secondary inoculum. Phytopathology®, 103(3), 261-267. https://doi.org/10.1094/phyto-08-12-0189-r

Agrios, G. N. 2005. Plant Pathology, 5th ed. Elsevier Academic Press, Amsterdam, the Netherlands.

Ali, M. E., Hudson, O., Hemphill, W. H., Brenneman, T. B., & Oliver, J. E. 2019. First report of resistance to pyraclostrobin, boscalid, and thiophanate-methyl in *Colletotrichum gloeosporioides* from blueberry in Georgia. Plant Health Progress, 20(4), 261-262.

Balzarini, M. G., González, L., Tablada, M., Casanoves, F., Di Rienzo, J. A., & Robledo, C. W. 2008. Infostat. Manual del usuario. Editorial Brujas, Córdoba, Argentina, 336.

Bautista-Montealegre, L. G., Bolaños-Benavides, M. M., Argüelles-Cárdenas, J. H., & Fischer, G. 2019. Fertilization with nitrogen, phosphorus, potassium and calcium in blackberry (*Rubus glaucus* Benth.): Effect on anthracnose under controlled conditions. Acta Agronómica, 68(3), 228-236. https://doi.org/10.15446/acag.v68n3.68337

Bell, S. R., Montiel, L. G. H., Estrada, R. R. G., & Martinez, P. G. 2021. Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. LWT-FOOD Science And Technology, 149. https://doi.org/10.1016/j.lwt.2021.112046

Castro, J. F., Cisterna-Oyarce, V., Carrasco-Fernández, J., Santelices, C., Muñoz, V., Guerra, M., Barra-Bucarei, L., France, A., & Millas, P. 2022. First report of *Colletotrichum fioriniae* causing anthracnose fruit rot on *Vaccinium corymbosum* in Chile. Plant Disease, 107(3), 959. https://doi.org/10.1094/PDIS-06-22-1340-PDN

Ciofini, A., Negrini, F., Baroncelli, R., & Baraldi, E. 2022. Management of Post-Harvest Anthracnose: Current Approaches and Future Perspectives. Plants, 11(14), 1856. https://www.mdpi.com/2223-7747/11/14/1856

De Silva, D. D., Ades, P. K., & Taylor, P. W. J. 2021. Pathogenicity of *Colletotrichum* species causing anthracnose of Capsicum in Asia. Plant Pathology, 70(4), 875-884. https://doi.org/https://doi.org/10.1111/ppa.13351

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. 2012. The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology, 13(4), 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Doi, K., Inoue, R., & Iwasaki, N. 2021. Seed weight mediates effects of pollen on berry weight, ripening, and anthocyanin content in highbush blueberry. Scientia Horticulturae, 288, 110313. https://doi.org/https://doi.org/10.1016/j.scienta.2021.110313

Dowling, M., Peres, N., Villani, S., & Schnabel, G. (2020). Managing *Colletotrichum* on Fruit Crops: A "Complex" Challenge. Plant Disease, 104(9), 2301-2316. https://doi.org/10.1094/PDIS-11-19-2378-FE

Ehlenfeldt, M. K., Polashock, J. J., Stretch, A. W., & Kramer, M. 2006. Leaf Disk Infection by *Colletotrichum acutatum* and Its Relation to Fruit Rot in Diverse Blueberry Germplasm. HortScience HortSci, 41(1), 270-271. https://doi.org/10.21273/hortsci.41.1.270

Fang, Y., Nunez, G. H., da Silva, M. N., Phillips, D. A., & Munoz, P. R. (2020). A Review for Southern Highbush Blueberry Alternative Production Systems. AGRONOMY-BASEL, 10(10). https://doi.org/10.3390/agronomy10101531

Feng, L., Sun, J., Jiang, Y., & Duan, X. (2022). Role of Reactive Oxygen species against pathogens in relation to postharvest disease of Papaya fruit. Horticulturae, 8(3), 205. https://www.mdpi.com/2311-7524/8/3/205

Forcelini, B. B., Gonçalves, F. P., & Peres, N. A. 2017. Effect of inoculum concentration and interrupted wetness duration on the development of anthracnose fruit rot of Strawberry. Plant Disease, 101(2), 372-377. https://doi.org/10.1094/pdis-08-16-1175-re

Gebrel, E., Gad, M. A., & Farouk, M. 2019. Response of some wheat cultivars to different nitrogen fertilizer rates and their relation to rust diseases. Egyptian Journal of Agronomy, 41(3), 243-254. https://doi.org/10.21608/agro.2019.14921.1169

González-Hernández, A. I., Fernández-Crespo, E., Scalschi, L., Hajirezaei, M.-R., von Wirén, N., García-Agustín, P., & Camañes, G. 2019. Ammonium mediated changes in carbon and nitrogen metabolisms induce resistance against *Pseudomonas syringae* in tomato plants. Journal of Plant Physiology, 239, 28-37. https://doi.org/https://doi.org/10.1016/j.jplph.2019.05.009

Guarnaccia, V., Martino, I., Brondino, L., Garibaldi, A., & Gullino, M. L. 2021. Leaf anthracnose and defoliation of blueberry caused by *Colletotrichum helleniense* in Northern Italy. Phytopathologia Mediterranea, 60(3), 479-491.

Guevara-Suarez, M., Cárdenas, M., Jiménez, P., Afanador-Kafuri, L., & Restrepo, S. 2022. *Colletotrichum* species complexes associated with crops in Northern South America: A Review. Agronomy, 12(3), 548. https://www.mdpi.com/2073-4395/12/3/548

Gupta, N., Debnath, S., Sharma, S., Sharma, P., & Purohit, J. 2017. Role of nutrients in controlling the plant diseases in sustainable agriculture. In V. S. Meena, P. K. Mishra, J. K. Bisht, & A. Pattanayak (Eds.), Agriculturally important microbes for sustainable agriculture: Volume 2: Applications in Crop Production and Protection (pp. 217-262). Springer Singapore. https://doi.org/10.1007/978-981-10-5343-6_8

Hagan, A. K., Bowen, K. L., Pegues, M., & Jones, J. 2014. Nitrogen rate and variety impact diseases and yield of sorghum for biofuel. Agronomy Journal, 106(4), 1205-1211. https://doi.org/https://doi.org/10.2134/agronj13.0483

Jacobs, M., Thompson, S., Platts, A. E., Body, M. J. A., Kelsey, A., Saad, A., Abeli, P., Teresi, S. J., Schilmiller, A., Beaudry, R., Feldmann, M. J., Knapp, S. J., Song, G.-q., Miles, T., & Edger, P. P. 2023. Uncovering genetic and metabolite markers associated with resistance against anthracnose fruit rot in northern highbush blueberry. Horticulture Research, 10(10). https://doi.org/10.1093/hr/uhad169

Joshi, R. 2018. A Review on Colletotrichum spp. Virulence mechanism against host plant defensive factors. Journal of Medicinal Plants Studies, 6, 64-67. https://doi.org/10.22271/plants.2018.v6.i6b.02

Lee, S. 2019. Climate change adaptive implementation assessment proposal for local governments utilizing vulnerability index. Journal of forest and environmental science, 35(1), 47-53.

Leoni, C., Bruzzone, J., Villamil, J. J., Martínez, C., Montelongo, M. J., Bentancur, O., & Conde-Innamorato, P. 2018. Percentage of anthracnose (*Colletotrichum acutatum* s.s.) acceptable in olives for the production of extra virgin olive oil. Crop Protection, 108, 47-53. https://doi.org/https://doi.org/10.1016/j.cropro.2018.02.013

Li, F., Matloob, M., Nzabanita, C., & Li, Y. 2021. Growth, sporulation and germination of *Verticillium alfalfae* on media. European Journal of Plant Pathology, 161(2), 383-395. https://doi.org/10.1007/s10658-021-02330-8

López-Moral, A., Agustí-Brisach, C., Lovera, M., Luque, F., Roca, L. F., Arquero, O., & Trapero, A. 2019. Effects of cultivar susceptibility, fruit maturity, leaf age, fungal isolate, and temperature on infection of almond by *Colletotrichum* spp. Plant Disease, 103(9), 2425-2432. https://doi.org/10.1094/pdis-12-18-2281-re

Lu, Y., Ma, D., Chen, X., & Zhang, J. 2018. A simple method for estimating field crop evapotranspiration from pot experiments. Water, 10(12), 1823. https://www.mdpi.com/2073-4441/10/12/1823

Mazzoni, L., Balducci, F., Di Vittori, L., Scalzo, J., Capocasa, F., Zhong, C.-F., Forbes-Hernandez, T. Y., Giampieri, F., Battino, M., & Mezzetti, B. 2020. Yield and nutritional quality of highbush blueberry genotypes trialled in a Mediterranean hot summer climate. Journal of the Science of Food and Agriculture, 100(9), 3675-3686. https://doi.org/https://doi.org/10.1002/jsfa.10403

Miles, T. D., Day, B., & Schilder, A. C. 2011. Identification of differentially expressed genes in a resistant versus a susceptible blueberry cultivar after infection by *Colletotrichum acutatum*. Molecular Plant Pathology, 12(5), 463-477. https://doi.org/10.1111/j.1364-3703.2010.00687.x

Miles, T. D., Gillett, J. M., Jarosz, A. M., & Schilder, A. M. C. 2013. The effect of environmental factors on infection of blueberry fruit by *Colletotrichum acutatum*. Plant Pathology, 62(6), 1238-1247. https://doi.org/https://doi.org/10.1111/ppa.12061

Miles, T. D., & Hancock, J. F. 2022. Inheritance of Resistance to Anthracnose Fruit Rot Caused by *Colletotrichum fioriniae* in Highbush Blueberry. International Journal Of Fruit Science, 22(1), 160-169. https://doi.org/10.1080/15538362.2021.2022567

Miles, T. D., Hancock, J. F., Callow, P., & Schilder, A. M. C. 2012. Evaluation of screening methods and fruit composition in relation to anthracnose fruit rot resistance in blueberries. Plant Pathology, 61(3), 555-566. https://doi.org/https://doi.org/10.1111/j.1365-3059.2011.02541.x

Moral, J., & Trapero, A. 2009. Assessing the susceptibility of Olive cultivars to anthracnose caused by *Colletotrichum acutatum*. Plant Disease, 93(10), 1028-1036. https://doi.org/10.1094/pdis-93-10-1028

Morkeliūnė, A., Rasiukevičiūtė, N., & Valiuškaitė, A. (2021). Meteorological conditions in a temperate climate for *Colletotrichum acutatum*, Strawberry pathogen distribution and susceptibility of different cultivars to anthracnose. Agriculture, 11(1), 80.

Muñoz-Espinoza, C., Di Genova, A., Correa, J., Silva, R., Maass, A., González-Agüero, M., Orellana, A., & Hinrichsen, P. 2016. Transcriptome profiling of grapevine seedless segregants during berry development reveals candidate genes associated with berry weight. BMC Plant Biology, 16(1), 104. https://doi.org/10.1186/s12870-016-0789-1

Muñoz-Vega, P., Paillán, H., Serri, H., Donnay, D., Sanhueza, C., Merino, E., & Hirzel, J. 2016. Effects of organic fertilizers on the vegetative, nutritional, and productive parameters of blueberries 'Corona', 'Legacy', and 'Liberty'. Chilean journal of agricultural research, 76, 201-212. http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-58392016000200010&nrm=iso

Muñoz, V., France, A., Uribe, H., & Hirzel, J. 2023. Nitrogen and Irrigation Rates Affected Leaf Phosphorus and Potassium Concentrations in Different Cultivars of Pot-Grown Blueberry. Journal of Soil Science and Plant Nutrition, 23(1), 965-973. https://doi.org/10.1007/s42729-022-01096-0

Nam, M. H., Jeong, S. K., Lee, Y. S., Choi, J. M., & Kim, H. G. 2006. Effects of nitrogen, phosphorus, potassium and calcium nutrition on strawberry anthracnose. Plant Pathology, 55(2), 246-249. https://doi.org/10.1111/j.1365-3059.2006.01322.x

Oliveira, P.B., Pinto, R.M., Mota, M. and Oliveira, C.M. 2017. Chilling effect on three highbush blueberry cultivars. Acta Hortic. 1180, 511-516. https://doi.org/10.17660/ActaHortic.2017.1180.72

Panth, M., Hassler, S. C., & Baysal-Gurel, F. 2020. Methods for management of soilborne diseases in crop production. Agriculture-Basel, 10(1). https://doi.org/10.3390/agriculture10010016

Phillips, D. A., Harmon, P. F., Olmstead, J. W., Peres, N. A., & Munoz, P. R. 2018. Screening for Susceptibility to Anthracnose Stem Lesions in Southern Highbush Blueberry. HortScience, 53(7), 920-924. https://doi.org/10.21273/hortsci12994-18

Piticar, A. 2019. Changes in agro-climatic indices related to temperature in Central Chile. International Journal of Biometeorology, 63(4), 499-510. https://doi.org/10.1007/s00484-019-01681-6

Polashock, J. J., Ehlenfeldt, M. K., Stretch, A. W., & Kramer, M. 2005. Anthracnose fruit rot resistance in blueberry cultivars. Plant Disease, 89(1), 33-38. https://doi.org/10.1094/PD-89-0033

Riolo, M., Pane, A., Santilli, E., Moricca, S., & Cacciola, S. O. 2023. Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose. Plant Pathology, 72(2), 255-267. https://doi.org/https://doi.org/10.1111/ppa.13652

Rodrigues, M. A., Coelho, V., Arrobas, M., Gouveia, E., Raimundo, S., Correia, C. M., & Bento, A. 2019. The effect of nitrogen fertilization on the incidence of olive fruit fly, olive leaf spot and olive anthracnose in two olive cultivars grown in rainfed conditions. Scientia Horticulturae, 256, 108658. https://doi.org/10.1016/j.scienta.2019.108658

Rodriguez-Saona, C., Vincent, C., & Isaacs, R. 2019. Blueberry IPM: Past successes and future challenges. Annual Review of Entomology, 64(1), 95-114. https://doi.org/https://doi.org/10.1146/annurev-ento-011118-112147

Roy, S., Nuckles, E., & Archbold, D. D. 2018. Effects of Phenolic Compounds on Growth of *Colletotrichum* spp. In Vitro. Current Microbiology, 75(5), 550-556. https://doi.org/10.1007/s00284-017-1415-7

Salotti, I., Ji, T., & Rossi, V. 2022. Temperature requirements of *Colletotrichum* spp. belonging to different clades. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.953760

Schmid, C. J., Clarke, B. B., & Murphy, J. A. 2017. Anthracnose severity and annual bluegrass quality as influenced by nitrogen source. Crop Science, 57(S1), S-285-S-292. https://doi.org/https://doi.org/10.2135/cropsci2016.06.0494

Sharma, S. 2020. Impacts of nitrogen on plant disease severity and plant defense mechanism. Fundamental and Applied Agriculture, 5(3), 303-314. https://doi.org/10.5455/faa.103334

Shukla, P. K., & Tarun, A. 2017. Anthracnose disease dynamics of mango orchards in relation to humid thermal ratio under subtropical climatic condition. Journal of Agrometeorology, 19(1), 56-61. https://doi.org/10.54386/jam.v19i1.756

Sun, Y., Wang, M., Mur, L. A. J., Shen, Q., & Guo, S. 2020. Unravelling the roles of nitrogen nutrition in plant disease defences. International Journal Of Molecular Science, 21(2), 572. https://doi.org/10.3390/ijms21020572

Swick, M.C., Koehler, T.M. and Driks, A. (2016). Surviving Between Hosts: Sporulation and Transmission. In Virulence Mechanisms of Bacterial Pathogens (eds I.T. Kudva, N.A. Cornick, P.J. Plummer, Q. Zhang, T.L. Nicholson, J.P. Bannantine and B.H. Bellaire). pp. 567-591. https://doi.org/10.1128/9781555819286.ch20

Talhinhas, P., Gonçalves, E., Sreenivasaprasad, S., & Oliveira, H. 2015. Virulence diversity of anthracnose pathogens (*Colletotrichum acutatum* and *C. gloeosporioides* species complexes) on eight olive cultivars commonly grown in Portugal. European Journal of Plant Pathology, 142(1), 73-83. https://doi.org/10.1007/s10658-014-0590-7

Uysal, A., & Kurt, Ş. 2017. Influence of inoculum density, temperature, wetness duration, and leaf age on infection and development of spinach anthracnose caused by the fungal pathogen *Colletotrichum spinaciae*. European Journal of Plant Pathology, 149(4), 1041-1052. https://doi.org/10.1007/s10658-017-1249-y

Verma, N., MacDonald, L., & Punja, Z. K. 2006. Inoculum prevalence, host infection and biological control of *Colletotrichum acutatum*: causal agent of blueberry anthracnose in British Columbia. Plant Pathology, 55(3), 442-450. https://doi.org/10.1111/j.1365-3059.2006.01401.x

Walters, D. R., & Bingham, I. J. 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Annals of Applied Biology, 151(3), 307-324. https://doi.org/10.1111/j.1744-7348.2007.00176.x

Wharton, P. S., & Schilder, A. C. 2008. Novel infection strategies of *Colletotrichum acutatum* on ripe blueberry fruit. Plant Pathology, 57(1), 122-134. https://doi.org/10.1111/j.1365-3059.2007.01698.x

Wichura, M. A., Koschnick, F., Jung, J., Bauer, S., & Wichura, A. 2024. Phenological growth stages of highbush blueberries (*Vaccinium* spp.): codification and description according to the BBCH scale. Botany, 102(11), 428-437. https://doi.org/10.1139/cjb-2024-0036

Xu, C. N., Zhou, Z. S., Wu, Y. X., Chi, F. M., Ji, Z. R., & Zhang, H. J. 2013. First report of stem and leaf anthracnose on blueberry caused by *Colletotrichum gloeosporioides* in China. Plant Disease, 97(6), 845-845. https://doi.org/10.1094/pdis-11-12-1056-pdn.

CAPITULO II.

Varietal susceptibility and effect of nitrogen fertilization on the incidence of anthracnose caused by *Colletotrichum fioriniae* on blueberry fruits

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ABSTRACT

The blueberry industry has become increasing competitive in recent years due to the advances achieved in a short term by new producing countries. The promotion of varietal replacement programs and the recent identification of *Colletotrichum fioriniae* in blueberries in Chile underscore the need for in-depth knowledge of the susceptibility of new commercial cultivars to anthracnose, and the relationship between disease prevalence and mineral fertilization. The aim of this research was to determine the varietal susceptibility to anthracnose and the effect of nitrogen fertilization on the incidence of this disease in blueberries. Eight blueberry cultivars and four nitrogen doses (0, 0.16, 0.32 and 0.48 g plant day^{-1}) were evaluated, while plants were inoculated at the green fruit stage, with *C. fioriniae* RGM 3330. For comparison, a non-inoculated control was also evaluated. Ripe fruits were collected at harvest to evaluate the incidence and severity of anthracnose. Additionally, leaves were inoculated *in vitro* with *C. fioriniae* and *Botrytis cinerea* to evaluate the ability of both fungi infecting vegetative tissues. Our results showed that mid-season ('Last

Call' and 'Legacy') and late-season cultivars ('Ochlockonee' and 'Victoria') were more susceptible to anthracnose, and the *C. fioriniae* infection was affected by environmental conditions. In the case of nitrogen fertilization, no significant effects were observed on the incidence and severity of anthracnose in fruits or vegetative tissues, but it influenced the necrotic area caused by *B. cinerea* on leaves. These findings provide relevant information for the development of phytosanitary programs for the management of *C. fioriniae* in blueberries.

INTRODUCTION

The consumption of blueberries (*Vaccinum* spp.) has increased dramatically in recent years, which has led to an increase in the cultivated area of this fruit worldwide (Fang et al., 2020; Rodriguez-Saona et al., 2019). Until recently, Chile was the world's largest blueberry exporter to the United States and Europe, reaching an export record of 117.640 tons during the 2020/21 season (IQconsulting, https://www.iqonsulting.com/yb/). However, the country's exports decreased by 20% during the 2022/23 season (ODEPA, 2023), being overtaken by Peru as the new leading exporter from the South Hemisphere. Furthermore, agricultural production has been affected by the reduction in fertilizer supplies after Russia's invasion of Ukraine, boosting prices and increasing production costs (Hebebrand et al., 2023). In this scenario, varietal replacement and intensive production systems are essential to improve yield and competitiveness of this small fruit crop (Fang et al., 2020; Mazzoni et al., 2020). In fact, evaluating the adaptation of new blueberry cultivars for early fruit production has become a matter of high interest, which underscores the need to compare yield potential with respect to traditionally-cultivated cultivars in Chile (Muñoz-Vega et al., 2016; Muñoz et al., 2023).

In parallel, the recent identification of *Colletotrichum fioriniae* (Marcelino & Gouli) Pennycook 2017 causing anthracnose in blueberry fruit in Chile (Castro et al., 2022) poses a relevant challenge to the industry, especially considering that postharvest fungi can reduce crop productivity by over 20% in this berry (Bell et al., 2021). Fungal species of the genus *Colletotrichum* are the causal agent of anthracnose, one of the most important postharvest diseases of blueberry in the United States (Bell et al., 2021; Dean et al., 2012). Anthracnose symptoms are observed in the field prior to harvest and during postharvest storage, causing sunken areas on the surface of ripe fruit, where acervuli develop and produce masses of orange-colored conidia that are visible on affected fruit (Wharton & Schilder, 2008). *Colletotrichum fioriniae* is a species belonging to the *C. acutatum* complex, which is characterized by causing infections at more moderate temperatures than other species of this genus (Salotti et al., 2022). This characteristic and the environmental variations associated with climate change have caused an increase in the occurrence of anthracnose in temperate climate areas (Lee, 2019; Morkeliūnė et al., 2021). In this sense, an in-depth study of anthracnose disease epidemiology and evaluation of varietal susceptibility are required to develop strategies for proper disease management. The control of this pathogen is mainly based on fungicide applications prior to and after harvest. However, isolates of *C. gloeosporioides* have shown resistance to the fungicides thiophanate-methyl (benzimidazole), pyraclostrobin (QoI group) and boscalid (DDHI group), which has been favored by the limited number of compounds available for the management of this disease (Ali et al., 2019). Recent research suggests that the use or development of varieties resistant to *Colletotrichum* spp. can be a potential strategy to manage anthracnose (Dowling et al., 2020; Panth et al., 2020). Unfortunately, there is insufficient scientific information regarding anthracnose-resistant blueberry varieties, characterized by early or mid-season fruit production (Miles & Hancock, 2022; Polashock et al., 2005). Previous studies have evaluated the susceptibility to anthracnose caused by *C. acutatum* in different blueberry genotypes, reporting results that indicate cultivars such as ‘Elliot’, ‘Legacy’, and ‘Brigitta Blue’ are resistant, while 'Jersey', 'Blue crop', 'Duke', and 'Flicker' as susceptible to the disease (Miles et al., 2011; Phillips et al., 2018; Polashock et al., 2005). To the best of the authors’ knowledge, no studies have reported on the susceptibility of new blueberry varieties to anthracnose. To prevent the development of epiphytes, which affects blueberry productivity, the use of new susceptible commercial varieties needs to be avoided (Phillips et al., 2018), also preventing an excessive use of fungicides for disease management.

Mineral nutrition can reduce or increase the development of diseases caused by different pathogens (Gupta et al., 2017; Walters & Bingham, 2007). Although anthracnose severity is affected by fertilization management, its relationship has not been clearly elucidated (Sun et al., 2020). Nam et al., (2006) determined that high concentrations of N and K increase anthracnose severity in strawberry, while other authors have described the opposite effect, reporting that symptoms decrease with increasing N doses in berry crops such as *Rubus glaucus* Benth (Bautista-Montealegre et al., 2019) or fruit trees such as *Olea europea* L. (Rodrigues et al., 2019).

Given the current scenario, the Chilean' blueberry industry needs to remain competitive by focusing on high quality and yield. Therefore, the aims of this research were to assess the susceptibility of new and traditional blueberry cultivars to anthracnose caused by *C. fioriniae*, recently reported in Chile; and to evaluate the effect of increasing doses of nitrogen fertilization on the severity of the disease on leaves and fruit in postharvest.

Materials and Methods

Plant material

Three-year-old blueberry plants of eight different cultivars were evaluated: *Vaccinium* sp. hybrid genotypes (including *V. corymbosum*, *V. darrowi*, and *V. ashei*), southern highbush blueberry 'Camellia', 'Suziblue' and 'Victoria'; northern highbush blueberry 'Cargo'. Duke' 'Last Call' and 'Legacy'; and the rabbiteye (*V. virginatum* Ait.) cv. 'Ochlockonee'. Regarding the analyzed cultivars, only 'Duke' and 'Legacy' have been reported as susceptible and resistant to anthracnose, respectively (Polashock et al., 2005). A total of 128 plants were used, considering 16 plants for each cultivar.

Experimental conditions

The experiment was established during the 2022/23 and 2023/24 seasons in the facilities of the Instituto de Investigaciones Agropecuarias (INIA), Quilamapu, Chillán, Ñuble region, Chile (36°35'44.2” S; 72°05'24.5” W), in a blueberry assay that was established in May 2019. Blueberry plants were grown outdoors, in 35 L pots containing a mixture of coconut fiber, peat, and perlite in a 2:2:1 (v/v) ratio, with a planting density of 20,000 plants ha^{-1}.

Nitrogen fertilization treatments

Plants were manually fertilized twice a week using a liquid solution of macro and micronutrients from October to April in each season (spring, summer and autumn) [Table 1]. The solution was supplemented with different doses of ammonium sulfate, considering the following treatments: a control treatment without application of nitrogen as ammonium sulfate (N0); 19.8 g N plant season^{-1} (N1x), corresponding to the recommended dose for a blueberry plant; 39.7 g N plant season^{-1} (N2x), corresponding to two times the recommended dose; and 59.9 g N plant season^{-1} (N3x) corresponding to three times the recommended dose (Table 1). Sixteen plants of each blueberry cultivar were used, considering four plants for each of the four nitrogen fertilization treatments. Nutrients P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B were applied as monoammonium phosphate, potassium sulfate, calcium nitrate, magnesium sulfate, and a micronutrient mixture containing magnesium oxide, B, Cu, Fe, Mn, Mo and Zn (Fetrilon Combi 2, COMPO EXPERT, Münster, Germany).

Agronomic management

The plants were pruned during May and June in both seasons, coinciding with the winter dormancy period. During the last week of August 2022 and the first week of October 2023, considering favorable environmental conditions and prior to the inoculation period, a single application of the fungicide fludioxonil (25%) and cyprodinil (37.5%) [Switch® 62. 5 WG, Syngenta, Chile] at dose of 80 g hL^{-1} was made with the objective of reducing possible flower infections by fungi. Weeds were controlled manually throughout the development of the experiment. Plants were irrigated from October to April by drip irrigation, using two emitters (Supertif PCND, Rivulis) per plant of 1.1 and 2.2 L h^{-1}. The plants of each cultivar were irrigated independently according to their water requirement using a Pro C programmer

(Hunter®, Hunter Engineering Company®, USA). The duration of each irrigation event was established weekly based on plant consumption of each cultivar by performing a water balance, considering changes in pot weights over 24 h to calculate the total water volume and control the moisture content in the pots (Lu et al., 2018).

Pathogen inoculation

An axenic monoconidial culture of the pathogen *C. fioriniae* strain RGM 3330 (Castro et al., 2022), obtained from the INIA Microbial Genetic Resource Bank collection (https://www.cchrgm.cl/), was used. Strain RGM 3330 was seeded on potato dextrose agar (APD) plates and incubated at 25 °C for 15 days to promote the formation of abundant spores. In a laminar flow chamber, spores were extracted by applying 3 mL of sterile distilled water over the fungus inside the plate and then its were suspended in the water using an L-loop until. The solution was extracted with a micropipette and filtered using sterile gauze into a 50 mL Falcon tube. The concentration the suspension was adjusted 1×10^6 conidia mL^{-1} by conidial counted with an hemocytometer and dilution. Pathogen inoculation was carried out between October and November, when the fruits of each variety were at the phenological macrostadium 75 (green fruit stage, about the size of a pea) according to Wichura et al. (2024), using an adaptation of the methodology described by Miles and Hancock (2022). For each season, the inoculation date was established when the plants of each cultivar reached the phenological stage already mentioned and was conducted on cultivars that simultaneously met the criteria for inoculation. Cultivars were divided into three groups: (I_1) 'Camellia' and 'Suziblue' (early flowering, between August 20 and October 2); (I_2) 'Duke', 'Last Call', and 'Legacy' (intermediate flowering, between September 2 and October 23); (I_3) 'Cargo', 'Ochlockonee', and 'Victoria' (late flowering, between September 20 and November 2). Between 3 to 5 mL of inoculum were applicated between 6 p.m. and 8 p.m., using a hand sprayer for each berry cluster present on the plant, trying to wet all the fruits in the clusters. Of the four plants of each variety and nitrogen fertilization treatment, three were inoculated with the pathogen *C. fioriniae*, and one plant was used as a control (without inoculation).

Environmental conditions during pathogen inoculation

Inoculation was carried out from October 13 to November 08, 2022 in the first season; and from October 19 to November 13, 2023 in the second season (Figures 1A and 1B, from the supplementary material). The onset of flowering and fruit development occurred later in the 2023/24 season, with delays of 7, 4 and 5 days in inoculation dates with respect to the 2022/23 season, for groups I_1, I_2, and I_3, respectively. When comparing mean temperatures during inoculation periods, there was a slight decrease during the second season. Specifically, there was a reduction in the average maximum air temperature of 1.5°C and 2.3°C in the period between October 10 and November 16, in 2022 and 2023 respectively. In addition, there were only three days with temperatures above 25°C, compared to 10 days recorded in 2022. On the other hand, there were intense precipitation events during the 2023 inoculation period, exceeding 10 mm of cumulative precipitation and reaching 46 mm two days prior to the third inoculation date. In contrast, precipitation events during the 2022 inoculation period, despite being similarly distributed when comparing to those in 2023, were generally less than 10 mm. Meteorological data were obtained from Chillan's weather station at www.agrometeorologia.cl and are available in Figure 1 of the supplementary material.

Evaluation of the incidence and severity of anthracnose caused by *C. fioriniae.*

At harvest, 50 fruits per plant were randomly selected and immediately taken to the laboratory, where they were placed in humid chambers equidistant from each other, and incubated for 5 days at room temperature between 22 and 24°C. Subsequently, the fruits were observed under a stereoscopic magnifying glass and those with presence of *C. fioriniae* or other postharvest fungi of the genera such as: *Botrytis*, *Cladosporium*, *Penicillium*, *Alternaria*, and *Aspergillus* were enumerated. Incidence was determined as the percentage of fruits colonized by each fungus with respect to the total number of fruits analyzed. Severity was evaluated using a visual scale of six categories (0 to 5), in which each value represents the proportion of fruit showing signs and symptoms of anthracnose in percentage, using an adaptation of the procedure proposed by Moral and Trapero (2009), as described in Table 1 and Figure 2 of the supplementary material.

Subsequently, based on the severity scale and considering the scores obtained, a severity index was calculated according to the formula used by Leoni et al. (2018):

$$Disease\ index: \frac{\sum(disease\ score\ x\ N°\ plants\ observed\ in\ this\ category)}{Maximum\ possible\ disease\ score\ x\ Total\ N°\ plants\ observed}\ x\ 100$$

Evaluation of leaf susceptibility under controlled laboratory conditions

Healthy leaves were selected from the middle third of shoots of the year that were collected from plants of each variety and nitrogen fertilization treatment. Using the methodology described by López-Moral et al. (2019), with some modifications, leaves were washed with potable water and then surface sterilized with 70% ethanol for 30 s, hypochlorite 1% for 1 min, 70% ethanol for 30 s, followed by three washes with sterile distilled water. For inoculation, three drops with 20 uL of a suspension of 10^{-6} conidia mL^{-1} of *C. fioriniae* and *Botrytis cinerea* Pers. were placed equidistantly on the surface of each leaf. The isolate of *B. cinerea* corresponded to strain FIT 321 obtained from blueberry fruits of cv. *Ochlockonee*, sampled during the experiment, which was morphologically identified and cultured as described for *C. fioriniae*. For each treatment, six leaves were inoculated with the pathogen and one leaf was inoculated with sterile distilled water as a control treatment. The wet chambers were incubated for 7 days at 24 ±3 °C, followed by photographic recording for evaluation. Photographs were analyzed using Image J software version 1.46r (USA) to obtain the lesion area and the total leaf area, and necrotic leaf area (NLA) was calculated as the ratio of the former to the latter. The experiment was conducted in each season.

Determination of leaf biomass

During May and June of 2022 and 2023, at the onset of leaf abscission or during plant dormancy, all leaves from each plant were harvested. The harvested leaves were oven-dried at 70 °C for 48 h or until a constant weight was reached. Subsequently, the dried leaves were weighed using an analytical balance to determine the total leaf biomass of each plant.

Experimental design and statistical analysis

Treatments were established using a completely randomized factorial design. The factors analyzed were cultivar, nitrogen fertilization treatments (N0 = 0; N1x = 19.8; N2x = 39.7; and N3x= 59.9 g plant season^{-1}), and season. Incidence, severity and necrotic leaf area (NLA) were analyzed by fitting an analysis of variance (ANOVA) model with three factors (cultivar, nitrogen rate, and season) and all their interactions, using the generalized linear mixed models (GLMMs) and the least significant difference (LSD) multiple comparison test (P <0.05), using INFOSTAT software (Balzarini et al., 2008). In addition, a principal component analysis was performed using 16 variables including: incidence of *C. fioriniae*, *Botrytis* spp., *Cladosporium* spp., *Penicillium* spp., *Alternaria* spp. and *Aspergillus* spp. on fruits; severity of anthracnose caused by *C. fioriniae* on fruits; environmental conditions during inoculation, including mean, minimum and maximum temperature, rainfall and relative humidity; flowering date (early, mid and late), leaf dry biomass and necrotic leaf area caused by *C. fioriniae* and *B. cinerea*. The analysis was performed using FactorMineR library and R statistical software according to Muñoz-Espinoza et al. (2016).

RESULTS

Significant differences were observed between cultivars (P< 0.05) in terms of incidence and severity of anthracnose, and NLA caused by *C. fioriniae*, but nitrogen fertilization treatments did not significantly influence infection by this pathogen. However, when evaluating NLA caused by *B. cinerea*, there was a significant effect of all sources of variation, including nitrogen fertilization treatments (Table 2). In the 2022/23 season, there was an interaction between cultivar and nitrogen fertilization for all the variables evaluated. Conversely, this was observed only in NLA caused by the two pathogens evaluated in the 2023/24 season (Table 2).

Evaluation of the incidence and severity of anthracnose caused by *C. fioriniae.*

A total of 11.210 fruits were analyzed during the entire evaluation period. In the 2022/23 season, although a minimum of 200 fruits was established for analysis (50 fruits per four replication), a lower number of fruits per plant was obtained in ‘Duke’ (107 fruits), ‘Legacy’ (150 fruits), ‘Last Call’ (122 fruits), and ‘Victoria’ (113 fruits). In the 2023/24 season, this situation occurred only in ‘Victoria’ (145 fruits). In this sense, it is important to indicate that ‘Victoria’ plants had defoliation and inadequate fruiting due to limited adaptation to the cultivation substrate, being excluded from the evaluations in the second season.

During the 2022/23 season, ‘Ochlockonee’, ‘Victoria’, ‘Duke’, and ‘Last Call’ had the highest anthracnose incidence in fruits, with values higher than 11.3%, compared to the rest of the cultivars evaluated, where the incidence of *C. fioriniae* did not exceed 5.5%. However, even lower infection levels were observed during the 2023/24 season, not exceeding 2.3% in all cultivars except for ‘Duke’ (Figure 1). This cultivar, which is considered susceptible to anthracnose, recorded similar incidence levels during the whole evaluation period, with values of 16.8% and 13.8% in the first and second seasons, respectively. ‘Camellia’ and ‘Suziblue’, cultivars with early flowering and harvest, consistently show a low disease incidence in fruits, with values between 0.33 and 0.5% in both seasons. On the other hand, disease incidence was reduced by more than 57% in cultivars with mid- to late-season flowering and harvest, ‘Legacy’ and ‘Last Call’, in the 2023/24 season with respect to the previous season. It is important to note that ‘Ochlockonee’, which is the last to be harvested among the cultivars evaluated, anthracnose incidence was 0% in the second season, representing a 100% reduction with respect to the 31.6% incidence (the highest infection level) observed in the 2022/23 season.

‘Legacy’, which is considered resistant to anthracnose, recorded a low disease incidence that did not exceed 5.5%. In general, there was no anthracnose incidence in the fruits of the plants in the control treatment (without inoculation of the pathogen) in both seasons. From the 3,000 control fruits assessed, which were not inoculated with *C. fioriniae*, only 7 and 12 fruits showed symptoms of the disease during the 2022/23 and 2023/24 seasons, respectively. During the 2022/23 season, the fruits with anthracnose collected from the plants in the control treatments belonged to ‘Legacy’, ‘Cargo’, ‘Last Call’, and ‘Ochlockonee’. However, during the 2023/24 season, the 12 fruits were only collected from ‘Duke’. The incidence of other postharvest fungi was low and on average did not exceed 3.1% and 3.8% for the first and second evaluation periods, respectively. ‘Camellia’ and ‘Suziblue’ showed a low anthracnose severity index, with values less than 1% in both seasons (Figure 2). During the 2022/23 season, ‘Ochlockonee’ had the highest severity index (31.6%), followed by ‘Victoria’ (19.0%), ‘Duke’ (11.4%) and ‘Last Call’ (6.2%), showing no significant differences between them (Figure 2). The decrease in anthracnose during the 2023/24 season resulted in lower disease severity, with values below 2% in all cultivars, except for ‘Duke’, which maintained similar values to those observed during the 2022/23 season, with an average of 9.1% for both evaluation period.

Susceptibility of leaves to inoculation by *C. fiorinia*e and *B. cinerea* under controlled conditions

The pathogen *C. fioriniae* was able to cause necrotic spots, sometimes coalescent and of variable size (0.01 to 23.4 cm^2) in all the evaluated cultivars, where acervuli and conidial masses developed on the surface of the leaves in a humid chamber under controlled conditions (Figure 3). During the 2022/23 season, the leaves of ‘Cargo’ plants were the most susceptible to inoculation with the pathogen, showing on average 18.8% necrosis with respect to the total leaf area, while ‘Duke’, being susceptible to anthracnose, was the second variety that presented the highest leaf necrosis, reaching an average value of 8.8% (Figure 4). There were no significant differences among the other six assessed cultivars, exhibiting a necrotic leaf area of less than 4.3%. Although *B. cinerea* was able to colonize leaves and cause lesions ranging in size from 0.01 to 6.19 cm^2, the necrotic area produced in this tissue was significantly lower than that caused by *C. fioriniae*. On average, the NLA caused by this pathogen was only 0.02 to 2.7%, and was significantly higher in 'Last Call' and 'Victoria'. On the other hand, unlike the results obtained with *C. fioriniae*, the NLA caused by *B. cinerea* was affected by nitrogen fertilization; N0 and N3x fertilization treatments caused a significant reduction in the average NLA values, compared to those obtained with the N2x treatment ($P < 0.05$; Figure 5).

Determination of leaf biomass

There were significant differences in leaf biomass production between nitrogen fertilization treatments during the 2022/23 season (S1), and significant differences between varieties in both evaluation seasons (S1 and S2). Although in the second season 2023/24, nitrogen fertilization did not affect leaf biomass, there was an interaction between cultivars and fertilization treatments ($P< 0.01$). In the first season, the nitrogen fertilization treatments were only different from the control treatment (without application of ammonium sulfate) (Figure 6). The leaf biomass production per plant was ranged between 33.6 and 172.8 g plant^{-1}, and the varieties with the highest production were values being observed in 'Camellia', 'Cargo', 'Ochlockonee' and 'Suziblue' during in the first 2022/23 season, while during the second season the leaf production was significantly higher; and in 'Camellia', 'Last Call', 'Ochlockonee' and 'Suziblue' in the 2023/24 season (Figure 7).

The principal component analysis, in which the influence of 16 variables on the susceptibility to anthracnose of 7 cultivars was assessed under different nitrogen doses and during two seasons, determined that components 1 and 2 together explained 39.4% of the total variability, with PC1 and PC2 responsible for 23.26% and 16.13%, respectively (Figure 8A). 'Victoria' was not considered in this statistical analysis because of the difficulties that this cultivar had to grow in the substrate cultivation. In Figure 8B shows that the variables with the greatest contribution to the variability between individuals were incidence and severity of anthracnose (C_fio and Sev_i), flowering date (Flowr), and environmental parameters such as minimum (T_min), maximum (T_max) temperature and relative humidity (RH). In addition, differences were observed between three groups of varieties in both evaluation periods. In the case of ‘Ochlockonee’ and ‘Cargo’, the variability between seasons was mainly determined by the incidence and severity of *C. fioriniae*, with positive and significant correlations with PC1 (r = 0.78, $P < 0.001$ and r = 0.80, $P < 0.001$, respectively). Regarding environmental parameters, minimum air temperature (T_min) had the greatest impact (r = 0.70, $P < 0.01$). In the case of ‘Duke’ and ‘Legacy’, the variation in maximum temperatures (T_max) and relative humidity (RH) between seasons were determinants in the separation of these cultivars, with these variables being significantly correlated with PC2 (r = 0.69, $P < 0.01$ and r = 0.72, $P < 0.01$, respectively). In parallel, flowering date (Flowr) showed a significant positive correlation with PC1 (r = 0.65, $P < 0.05$), indicating that the flowering period also contributed to the seasonal differences between ‘Duke’ and ‘Legacy’. Finally, for ‘Suziblue’ and ‘Camellia’, leaf dry weight (LDW) and necrotic leaf area caused by *C. fioriniae* (NLA_C) were the main variables resulting in differences between seasons for these cultivars as both were positively correlated with PC2 (r = 0.67, $P < 0.05$ and r = 0.63, $P < 0.05$, respectively). Likewise, mean air temperature (T_air) also contributed to the variability between seasons (r = 0.68, $P < 0.05$), highlighting the relevance that environmental conditions had during pathogen infection in each season.

Discussion

Differences in fruit susceptibility to infection by *C. fioriniae* were found among the eight different blueberry cultivars evaluated, some of which have been suggested for varietal replacement in Chile. Although there were differences between both seasons, the early harvest cultivars ‘Camellia’ and ‘Suziblue’ showed an average incidence of less than 0.5%, while mid- and late-harvest cultivars ‘Duke’, ‘Victoria’ and ‘Ochlockonee’ reached had an incidence between 13.8 and 44.5%. On the other hand, this research determined that nitrogen fertilization had no effect on the incidence and severity of anthracnose caused by *C. fioriniae* on fruit and leaves. The variability observed between different groups of varieties in the two evaluation seasons was mainly explained by the environmental conditions during inoculation, flowering date, and susceptibility to *C. fioriniae*. Nevertheless, in the evaluations carried out to assess the colonization of *C. fioriniae* and *B. cinerea* in detached leaves under *in vitro* conditions, differences were observed in the susceptibility to *B. cinerea* of these tissues between the evaluated cultivars associated with N fertilization. Causing more necrotic symptoms in leaves fertilized with the N2x (39.7 g plant^{-1}) and N1x (19.8 g plant^{-1}) treatments, compared to the control treatment (not treated with ammonium sulphate). This variable had no effect on leaf necrosis caused by *C. fioriniae*.

Previous works have evaluated the susceptibility to anthracnose of a large number of blueberry varieties (Polashock et al., 2005). This research included varieties such as ‘Camellia’, ‘Suziblue’, ‘Cargo’, ‘Victoria’ that are currently on the market, but whose resistance to the disease has not been elucidated yet. In all the assessed cultivars, there were fruits that showed symptoms and signs of *C. fioriniae*, indicating that none of the eight cultivars evaluated was immune to this disease. This finding agrees with Miles and Hancock (2022), who conducted a study in three blueberry cultivars with known resistance profiles (‘Bluecrop’, ‘Elliott’, and ‘Jersey’) and progeny from 16 crosses of parents with varying levels of susceptibility and reported no immunity in fruits. Furthermore, Polashock et al. (2005) determined that the incidence of anthracnose fluctuates between approximately 10% and 90% among different blueberry cultivars, with a proportion of diseased fruit of 44% in ‘Duke’ and 10% in ‘Legacy´, which suggests that the former is an anthracnose susceptible genotype, and the latter is resistant to the disease. These results coincide with those obtained in our work; although the incidence in these cultivars was lower in our evaluation, infection was proportionally higher in ‘Duke’ compared to ‘Legacy’ during both experimental seasons.

Early-harvest cultivars ‘Camellia’ and ‘Suziblue’ showed a low disease incidence, but more information is required to determine whether this response is related to the resistance of these genotypes or to the environmental conditions prevailing during fruit development. Cultivars with low chilling requirements bloom earlier in the season and their fruits develop in a period of lower temperatures compared to cultivars with higher chilling hour requirement (Oliveira et al., 2017). This characteristic could explain why mid- and late-harvest cultivars, such as ‘Duke’, ‘Last Call’ and ‘Victoria’, showed a higher incidence of anthracnose in fruits. Under favorable conditions and in the presence of inoculum, the disease affected particularly cultivars that had a later bloom in the season as observed in ‘Ochlockonee’. In this sense, Miles et al. (2012) indicated that due to differences in ripening dates and chilling requirements in blueberries, it is difficult to compare anthracnose susceptibility between different cultivars. Flowering time affects fruit infection, which can lead to ‘field resistance’ or escape, resulting in an unreliable indicator of susceptibility. However, even though ‘Duke’ and ‘Legacy’ were inoculated at the same time, they exhibited differences of about 11% in anthracnose incidence, which was consistent in both evaluation periods and confirms the resistance of ‘Legacy’ to *C. fioriniae*.

Resistance to *Colletotrichum* species has been associated with different defense mechanisms, including the production of phenolic compounds, which inhibit mycelial growth (Miles et al., 2011; Roy et al., 2018); production of reactive oxygen species (ROS), which inhibit conidial germination (Feng et al., 2022); and activation of genes encoding acidic chitinase, protease inhibitors, and polygalacturonase inhibitory proteins (PGIP) to prevent the penetration of the fungus (Joshi, 2018). Furthermore, blueberry cultivar resistance has been expressed in both green and ripe fruits (Miles et al., 2011; Wharton & Schilder, 2008). Recently, progress has been made in determining the role of flavonoid compounds and identifying molecular markers associated with *C. fioriniae* resistance in blueberries (Jacobs et al., 2023). Therefore, chemical and physical characterization of the fruits of resistant cultivars, and the identification of the metabolic pathways and genes associated with the resistance are tools to advance in the selection of anthracnose-resistant cultivars in blueberries.

Like disease incidence, severity varies from year to year influenced by environmental factors (Shukla & Tarun, 2017; Uysal & Kurt, 2017). In our research, disease severity was higher in mid- and late-flowering cultivars, such as ‘Victoria’ and ‘Ochlockonee’, with respect to early-flowering cultivars, such as ‘Camellia’ and ‘Suziblue’, in the 2022/23 season; however, all evaluated cultivars recorded values that did not exceed 2% in the following season, with the exception of ‘Duke’ that recorded 9.1%. Nitrogen fertilization had no significant effect on anthracnose severity, which agrees with previous findings reported in the literature (Hagan et al., 2014; Rodrigues et al., 2019), but it had an impact on canopy growth (Figure 6), suggesting that other factors play a more important role in disease development (Miles et al., 2013; Rodrigues et al., 2019). Contrary to our results, studies show that there is an effect of nitrogen fertilization on the incidence of anthracnose severity, however, there are discrepancies between research works (Rodrigues et al., 2019; Schmid et al., 2017). This is due to the complex relationship between N rates (or N forms) and plant diseases. It has been indicated that N has negative effects on physical defenses and the production of phytoalexins but positive effects on the production of enzymes and proteins that are related to local and systemic defense responses in the plant (Sun et al., 2020). This does not agree with our results as a significant interaction was observed between nitrogen dose and cultivar, being consistent with Gebrel et al. (2019). In bioassays carried out under controlled conditions by De Silva et al. (2021), an interaction was determined between inoculation method, susceptibility of the host, and fruit maturity on the infection and rate of lesion development, caused by different species of *Colletotrichum*. However, it is necessary to consider that fruit physiological maturity affects the aggressiveness of *Colletotrichum* spp. (Riolo et al., 2023). The evaluation in blueberry fruits is difficult to estimate, since berries have a different ripening rate in a single fruiting branch or even in the same bunch (Doi et al., 2021). Likewise, if we consider that the pathogen growth rate is the same, the size of the fruits will affect the time required by the pathogen to completely cover the fruit (Miles et al., 2011). Therefore, these variables must be considered to adequately relate varietal susceptibility and nitrogen fertilization.

Among the environmental conditions that explain the annual variations in the incidence and severity of anthracnose, temperatures play a key role (Miles et al., 2013). The optimal temperatures for the germination of *Colletotrichum* conidia are between 20 and 25 °C, with a range between 5 and 36 °C (Salotti et al., 2022). Other factors associated with the risk of infection are the intensity, humidity on the surface of the leaves, and light intensity (Ciofini et al., 2022). In this regard, it has been determined that a minimum of 6 h of humidity is required for the formation of a melanized appressorium by the fungus (Miles et al., 2013). These findings coincide with the results obtained with the PCA regarding the influence that air temperature and relative humidity had on the variability observed between different groups of varieties between seasons. These two environmental variables are directly related to flowering date and are determining factors during the fungal infection period. Based on this, we can indicate that in both seasons, the plants of group I_1 (early-flowering cultivars 'Camellia' and 'Suziblue') were inoculated on days without precipitation, with an average temperature close to 10 °C (supplementary material, Figure 1A). These conditions would not favor the development of appressoria or *Colletotrichum* conidia since conidial infection does not occur at temperatures below 10 °C in the species of the *C. accutatum* complex (Salotti et al., 2022). In the 2022/23 season, plants of groups I2 ('Duke', 'Legacy' and 'Last Call') and I3 ('Cargo', 'Victoria' and 'Ochlockonee') were inoculated under conditions considered appropriate for infection, because the mean and maximum temperatures were within the ranges that allow the development of appressoria (Miles et al., 2013). In addition, there were precipitation events during four consecutive days after the inoculation date, which could have caused humidity conditions that favored the pathogen infection (Forcelini et al., 2017; Morkeliūnė et al., 2021). When humidity remains high for more than 12-18 h, infection caused by *C. accutatum* increases in immature blueberries (Miles et al., 2013). In the present study, precipitation events in 2023/24 season occurred prior to inoculation of these groups, and inoculation was carried out at temperatures lower than those recorded the previous season. In particular, inoculation for group I3 was carried out with average temperatures close to 10°C (supplementary material, Figure

1B), which is a hindrance to the infection by this pathogen and would explain the low severity observed in the evaluations of that season.

Colletotrichum species are considered pathogens associated with tropical and subtropical climates, but they are also distributed in temperate climates (Guevara-Suarez et al., 2022; Phillips et al., 2018). Because *C. acutatum* can cause infections at lower temperatures, this disease has spread in temperate areas, beginning to cause more recurrent epiphytes in crops such as strawberries (Morkeliūnė et al., 2021). In Chile, agriculture is mainly developed in the central zone (between 33 °00' and 44 °00' S latitude), which is characterized by a temperate climate and where precipitation gradually increases from north to south, while temperatures decrease in the same direction (Piticar, 2019). As high temperature and humidity are not likely to occur simultaneously, the absence of environmental conditions that favor infections caused by *Colletotrichum* spp. would prevent the development and dissemination of anthracnose in Chile. Therefore, the environmental conditions of each season will be determining factors in the potential risk of disease development.

In blueberry, although *Colletotrichum* sp. mainly affects fruits, causing rot during and after harvest (Bell et al., 2021), there are reports on blueberry cultivars particularly susceptible to anthracnose, in which *Colletotrichum* species can affect both fruits and vegetative tissues, causing lesions in green stems and leaves, resulting in defoliation and loss of yield (Guarnaccia et al., 2021; Phillips et al., 2018; Xu et al., 2013). Leaf susceptibility is a relevant parameter because leaves, along with other vegetative tissues, constitute a reservoir and source of inoculum for this pathogen (Verma et al., 2006). Coinciding with our results, a previous study by Ehlenfeldt et al. (2006), regarding leaf susceptibility to infection by *Colletotrichum* in blueberry cultivars, established significant differences between cultivars in the size of the lesions caused by the fungus, with leaf necrosis ranging from 8 to 79% and averaging 32%. However, the authors did not observe a correlation between leaf lesions and incidence or severity of anthracnose in the fruits. Similarly, the results of our study revealed that there were no correlations between leaf damage and disease susceptibility in the fruits of the eight varieties evaluated. As determined by

Ehlenfeldt et al. (2006), our results confirm that leaf necrosis cannot be considered a predictive factor to determine susceptibility of fruits to this disease. On the other hand, levels of necrosis in leaves inoculated with *C. fioriniae* ranged from 14.8 to 100% and averaged 4.8%, differing from the results of Guarnaccia et al. (2021), who reported that disease severity in leaves was significantly higher in ‘Last Call’ compared to that in ‘Duke’. In the present study, ‘Duke’ was more susceptible than ‘Last Call’, but this discrepancy can be explained by that fact that the authors used *C. helleniense*, a fungus associated with the *C. gloeosporioides* species complex. In this sense, a study that assessed the severity of anthracnose caused by different species of the *C. acutatum* and *C. gleosporoides* complexes in eight olive cultivars, determined that the isolates showed diverse virulence and interaction between pathogens and cultivars, suggesting differences in conidial germination and appressoria formation during early events in host-pathogen recognition (Talhinhas et al., 2015). This virulence feature in *Colletotrichum* species could explain the contrasting results regarding the susceptibility of ‘Last Call’ and ‘Duke’ as reported by Guarnaccia et al. (2021) and what was observed in the present study.

Depending on the pathogen, a single nutrient can have opposite effects in different environments, increasing the occurrence of one disease and reducing the occurrence of others (Agrios, 2005). In general, nitrogen fertilization had no significant effect on NLA when inoculating with *C. fioriniae*, but there was an impact on it when inoculating leaves with *B. cinerea*. These results suggest that these pathogens respond differentially to nitrogen fertilization. In this regard, the level of nitrogen fertilization of the host plant has a significant impact on the abundance and pathogenicity of the secondary inoculum produced by *B. cinerea* (Abro et al., 2013). Furthermore, some pathogens have specific carbon and nitrogen requirements for sporulation, while this process can also be triggered by nutrient depletion (Abro et al., 2013; Li et al., 2021; Swick et al., 2016). In the case of ammonium, it is suggested that this molecule can cause a nutrient imbalance, which causes a reduction of the intermediates of the tricarboxylic acid cycle, allowing the activation of the induced systemic response and explaining the defense response of the host plant against the pathogen (González-Hernández et al. (2019). Therefore, determining how different nitrogen concentrations in the plant influence the expression of defense genes in blueberry varieties is a topic that should be studied.

Increased N supply may induce greater susceptibility to diseases due to changes in canopy structures that increase crop density, generating a microclimate that could favor pathogens (Sharma, 2020). Although nitrogen fertilization caused a significant increase in leaf biomass (108.2 g plant^{-1} in average) compared to the control treatment without application of ammonium sulfate (83.5 g plant^{-1}) (Figure 6) in the 2022/23 season, leaf biomass did not have a significant contribution to the total variability of the different cultivars and nitrogen fertilization treatments according to the PCA. This result suggests that changes in leaf biomass were not a relevant variable in the incidence and severity of anthracnose in the present research. On the other hand, the source of N can have an important effect on the severity of anthracnose. The application of potassium nitrate and calcium nitrate have shown to reduce the disease compared to acidifying sources of N (Schmid et al., 2017), such as the ammonium sulphate used in this study. This substrate acidification would be related to changes in soil pH that could induce deficiency of other nutrients and the stress that this chemical variable of the soil could cause in the plant, favoring a greater susceptibility to the pathogen (Schmid et al., 2017). In the present research, ammonium sulphate was used because this is the most widely used nitrogen fertilizer in blueberries, since it maintains the acidic soil requirements required for the production of this berry. For this reason, evaluating how different concentrations of nitrogen obtained from different nitrogen sources influence the expression of anthracnose in blueberries can be a variable to consider when evaluating varieties that present intermediate resistance to *C. fioriniae*.

Finally, the obtained results suggest that, in the presence of inoculum, environmental conditions during flowering, and fruit development are determinants in the incidence and severity of anthracnose caused by *C. fioriniae* in blueberry varieties of the species *V. corymbosum* hybrid, *V. corymbosum*, and *V. virguinatum*, which have different flowering times. Cultivars such as ‘Camellia’ and ‘Suziblue’, characterized by a lower chilling requirement and early flowering, showed a lower susceptibility to anthracnose in both evaluation periods. In contrast, mid- and late-harvest cultivars such as 'Duke', 'Last Call', 'Victoria' and 'Ochlockonee' showed a higher susceptibility to the disease under environmental conditions that can lead to infection by the pathogen. In addition, nitrogen fertilization had no impact on the incidence or severity of *C. fioriniae* in the cultivars evaluated. On the other hand, both *C. fioriniae* and *B. cinerea* managed to colonize blueberry leaves and cause necrotic spots, but only *B. cinerea* showed an increase in leaf necrosis associated with an increase in nitrogen fertilization.

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Literature cited

Abro, M. A., Lecompte, F., Bryone, F., & Nicot, P. C. 2013. Nitrogen fertilization of the host plant influences production and pathogenicity of *Botrytis cinerea* secondary inoculum. Phytopathology, 103(3), 261-267. https://doi.org/10.1094/phyto-08-12-0189-r

Agrios, G. N. 2005. Plant Pathology, 5th ed. Elsevier Academic Press, Amsterdam, the Netherlands.

Ali, M. E., Hudson, O., Hemphill, W. H., Brenneman, T. B., & Oliver, J. E. 2019. First report of resistance to pyraclostrobin, boscalid, and thiophanate-methyl in *Colletotrichum gloeosporioides* from blueberry in Georgia. Plant Health Prog. 20(4), 261-262.

Balzarini, M. G., González, L., Tablada, M., Casanoves, F., Di Rienzo, J. A., & Robledo, C. W. 2008. Infostat. Manual del usuario. Editorial Brujas, Córdoba, Argentina, 336.

Bautista-Montealegre, L. G., Bolaños-Benavides, M. M., Argüelles-Cárdenas, J. H., & Fischer, G. 2019. Fertilization with nitrogen, phosphorus, potassium and calcium in blackberry (*Rubus glaucus* Benth.): Effect on anthracnose under controlled conditions. Acta Agron. 68(3), 228-236. https://doi.org/10.15446/acag.v68n3.68337

Bell, S. R., Montiel, L. G. H., Estrada, R. R. G., & Martinez, P. G. 2021. Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. LWT-Food Sci Technol. 149. https://doi.org/10.1016/j.lwt.2021.112046

Castro, J. F., Cisterna-Oyarce, V., Carrasco-Fernández, J., Santelices, C., Muñoz, V., Guerra, M., Barra-Bucarei, L., France, A., & Millas, P. 2022. First report of *Colletotrichum fioriniae* causing anthracnose fruit rot on *Vaccinium corymbosum* in Chile. Plant Dis. 107(3), 959.

Ciofini, A., Negrini, F., Baroncelli, R., & Baraldi, E. 2022. Management of Post-Harvest Anthracnose: Current Approaches and Future Perspectives. Plants, 11(14), 1856. https://www.mdpi.com/2223-7747/11/14/1856

De Silva, D. D., Ades, P. K., & Taylor, P. W. J. 2021. Pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum* in Asia. Plant Pathol. 70(4), 875-884. https://doi.org/https://doi.org/10.1111/ppa.13351

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 13(4), 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Doi, K., Inoue, R., & Iwasaki, N. 2021. Seed weight mediates effects of pollen on berry weight, ripening, and anthocyanin content in highbush blueberry. Sci Hortic, 288, 110313. https://doi.org/https://doi.org/10.1016/j.scienta.2021.110313

Dowling, M., Peres, N., Villani, S., & Schnabel, G. (2020). Managing *Colletotrichum* on Fruit Crops: A "Complex" Challenge. Plant Dis, 104(9), 2301-2316. https://doi.org/10.1094/PDIS-11-19-2378-FE

Ehlenfeldt, M. K., Polashock, J. J., Stretch, A. W., & Kramer, M. 2006. Leaf disk infection by *Colletotrichum acutatum* and its relation to fruit rot in diverse blueberry germplasm. HortScience, 41(1), 270-271. https://doi.org/10.21273/hortsci.41.1.270

Fang, Y., Nunez, G. H., Silva, M. N. d., Phillips, D. A., & Munoz, P. R. 2020. A Review for Southern Highbush Blueberry alternative production systems. Agronomy, 10(10), 1531. https://doi.org/10.3390/agronomy10101531

Feng, L., Sun, J., Jiang, Y., & Duan, X. 2022. Role of Reactive Oxygen species against pathogens in relation to postharvest disease of Papaya fruit. Horticulturae, 8(3), 205. https://www.mdpi.com/2311-7524/8/3/205

Forcelini, B. B., Gonçalves, F. P., & Peres, N. A. 2017. Effect of inoculum concentration and interrupted wetness duration on the development of anthracnose fruit rot of Strawberry. Plant Dis. 101(2), 372-377. https://doi.org/10.1094/pdis-08-16-1175-re

Gebrel, E., Gad, M. A., & Farouk, M. 2019. Response of some wheat cultivars to different nitrogen fertilizer rates and their relation to rust diseases. Egyptian Journal of Agronomy, 41(3), 243-254. https://doi.org/10.21608/agro.2019.14921.1169

González-Hernández, A. I., Fernández-Crespo, E., Scalschi, L., Hajirezaei, M.-R., von Wirén, N., García-Agustín, P., & Camañes, G. 2019. Ammonium mediated changes in carbon and nitrogen metabolisms induce resistance against *Pseudomonas syringae* in tomato plants. J Plant Physiol. 239, 28-37. https://doi.org/https://doi.org/10.1016/j.jplph.2019.05.009

Guarnaccia, V., Martino, I., Brondino, L., Garibaldi, A., & Gullino, M. L. 2021. Leaf anthracnose and defoliation of blueberry caused by *Colletotrichum helleniense* in Northern Italy. Phytopathol Mediterr. 60(3), 479-491.

Guevara-Suarez, M., Cárdenas, M., Jiménez, P., Afanador-Kafuri, L., & Restrepo, S. 2022. *Colletotrichum* species complexes associated with crops in Northern South America: A Review. Agronomy, 12(3), 548. https://www.mdpi.com/2073-4395/12/3/548

Gupta, N., Debnath, S., Sharma, S., Sharma, P., & Purohit, J. 2017. Role of nutrients in controlling the plant diseases in sustainable agriculture. In V. S. Meena, P. K. Mishra, J. K. Bisht, & A. Pattanayak (Eds.), Agriculturally important microbes for sustainable agriculture: Volume 2: Applications in Crop Production and Protection (pp. 217-262). Springer Singapore. https://doi.org/10.1007/978-981-10-5343-6_8

Hagan, A. K., Bowen, K. L., Pegues, M., & Jones, J. 2014. Nitrogen rate and variety impact diseases and yield of sorghum for biofuel. Agron J. 106(4), 1205-1211. https://doi.org/https://doi.org/10.2134/agronj13.0483

Jacobs, M., Thompson, S., Platts, A. E., Body, M. J. A., Kelsey, A., Saad, A., Abeli, P., Teresi, S. J., Schilmiller, A., Beaudry, R., Feldmann, M. J., Knapp, S. J., Song, G.-q., Miles, T., & Edger, P. P. 2023. Uncovering genetic and metabolite markers associated with resistance against anthracnose fruit rot in northern highbush blueberry. Hortic Res. 10(10). https://doi.org/10.1093/hr/uhad169

Joshi, R. 2018. A Review on Colletotrichum spp. Virulence mechanism against host plant defensive factors. J Med Plants Stud. 6, 64-67. https://doi.org/10.22271/plants.2018.v6.i6b.02

Lee, S. 2019. Climate change adaptive implementation assessment proposal for local governments utilizing vulnerability index. J For Environ Sci, 35(1), 47-53.

Leoni, C., Bruzzone, J., Villamil, J. J., Martínez, C., Montelongo, M. J., Bentancur, O., & Conde-Innamorato, P. 2018. Percentage of anthracnose (*Colletotrichum acutatum* s.s.) acceptable in olives for the production of extra virgin olive oil. Crop Prot. 108, 47-53. https://doi.org/https://doi.org/10.1016/j.cropro.2018.02.013

Li, F., Matloob, M., Nzabanita, C., & Li, Y. 2021. Growth, sporulation and germination of *Verticillium alfalfae* on media. Eur J Plant Pathol. 161(2), 383-395. https://doi.org/10.1007/s10658-021-02330-8

López-Moral, A., Agustí-Brisach, C., Lovera, M., Luque, F., Roca, L. F., Arquero, O., & Trapero, A. 2019. Effects of cultivar susceptibility, fruit maturity, leaf age, fungal isolate, and temperature on infection of almond by *Colletotrichum* spp. Plant Dis, 103(9), 2425-2432. https://doi.org/10.1094/pdis-12-18-2281-re

Lu, Y., Ma, D., Chen, X., & Zhang, J. 2018. A simple method for estimating field crop evapotranspiration from pot experiments. Water, 10(12), 1823. https://www.mdpi.com/2073-4441/10/12/1823

Mazzoni, L., Balducci, F., Di Vittori, L., Scalzo, J., Capocasa, F., Zhong, C.-F., Forbes-Hernandez, T. Y., Giampieri, F., Battino, M., & Mezzetti, B. 2020. Yield and nutritional quality of highbush blueberry genotypes trialled in a Mediterranean hot summer climate. J Sci Food Agric. 100(9), 3675-3686. https://doi.org/https://doi.org/10.1002/jsfa.10403

Miles, T. D., Day, B., & Schilder, A. C. 2011. Identification of differentially expressed genes in a resistant versus a susceptible blueberry cultivar after infection by *Colletotrichum acutatum*. Mol Plant Pathol, 12(5), 463-477. https://doi.org/10.1111/j.1364-3703.2010.00687.x

Miles, T. D., Gillett, J. M., Jarosz, A. M., & Schilder, A. M. C. 2013. The effect of environmental factors on infection of blueberry fruit by *Colletotrichum acutatum*. Plant Pathol. 62(6), 1238-1247. https://doi.org/https://doi.org/10.1111/ppa.12061

Miles, T. D., & Hancock, J. F. 2022. Inheritance of Resistance to Anthracnose Fruit Rot Caused by *Colletotrichum fioriniae* in Highbush Blueberry. Int J Fruit Sci. 22(1), 160-169. https://doi.org/10.1080/15538362.2021.2022567

Miles, T. D., Hancock, J. F., Callow, P., & Schilder, A. M. C. 2012. Evaluation of screening methods and fruit composition in relation to anthracnose fruit rot resistance in blueberries. Plant Pathol. 61(3), 555-566. https://doi.org/https://doi.org/10.1111/j.1365-3059.2011.02541.x

Moral, J., & Trapero, A. 2009. Assessing the susceptibility of Olive cultivars to anthracnose caused by *Colletotrichum acutatum*. Plant Dis. 93(10), 1028-1036. https://doi.org/10.1094/pdis-93-10-1028

Morkeliūnė, A., Rasiukevičiūtė, N., & Valiuškaitė, A. (2021). Meteorological conditions in a temperate climate for *Colletotrichum acutatum*, Strawberry pathogen distribution and susceptibility of different cultivars to anthracnose. Agriculture, 11(1), 80.

Muñoz-Espinoza, C., Di Genova, A., Correa, J., Silva, R., Maass, A., González-Agüero, M., Orellana, A., & Hinrichsen, P. 2016. Transcriptome profiling of grapevine seedless segregants during berry development reveals candidate genes associated with berry weight. BMC Plant Biol. 16(1), 104. https://doi.org/10.1186/s12870-016-0789-1

Muñoz-Vega, P., Paillán, H., Serri, H., Donnay, D., Sanhueza, C., Merino, E., & Hirzel, J. 2016. Effects of organic fertilizers on the vegetative, nutritional, and productive parameters of blueberries 'Corona', 'Legacy', and 'Liberty'. Chil J Agric Res. 76, 201-212. http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-58392016000200010&nrm=iso

Muñoz, V., France, A., Uribe, H., & Hirzel, J. 2023. Nitrogen and Irrigation Rates Affected Leaf Phosphorus and Potassium Concentrations in Different Cultivars of Pot-Grown Blueberry. J Soil Sci Plant Nutr. 23(1), 965-973. https://doi.org/10.1007/s42729-022-01096-0

Nam, M. H., Jeong, S. K., Lee, Y. S., Choi, J. M., & Kim, H. G. 2006. Effects of nitrogen, phosphorus, potassium and calcium nutrition on strawberry anthracnose. Plant Pathol. 55(2), 246-249. https://doi.org/10.1111/j.1365-3059.2006.01322.x

Oliveira, P.B., Pinto, R.M., Mota, M. and Oliveira, C.M. 2017. Chilling effect on three highbush blueberry cultivars. Acta Hortic. 1180, 511-516. https://doi.org/10.17660/ActaHortic.2017.1180.72

Panth, M., Hassler, S. C., & Baysal-Gurel, F. 2020. Methods for management of soilborne diseases in crop production. Agriculture, 10(1), 16. https://doi.org/10.3390/agriculture10010016

Phillips, D. A., Harmon, P. F., Olmstead, J. W., Peres, N. A., & Munoz, P. R. 2018. Screening for Susceptibility to Anthracnose Stem Lesions in Southern Highbush Blueberry. HortScience, 53(7), 920-924. https://doi.org/10.21273/hortsci12994-18

Piticar, A. 2019. Changes in agro-climatic indices related to temperature in Central Chile. Int J Biometeorol. 63(4), 499-510. https://doi.org/10.1007/s00484-019-01681-6

Polashock, J. J., Ehlenfeldt, M. K., Stretch, A. W., & Kramer, M. 2005. Anthracnose fruit rot resistance in blueberry cultivars. Plant Dis. 89(1), 33-38. https://doi.org/10.1094/PD-89-0033

Riolo, M., Pane, A., Santilli, E., Moricca, S., & Cacciola, S. O. 2023. Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose. Plant Pathol. 72(2), 255-267. https://doi.org/https://doi.org/10.1111/ppa.13652

Rodrigues, M. A., Coelho, V., Arrobas, M., Gouveia, E., Raimundo, S., Correia, C. M., & Bento, A. 2019. The effect of nitrogen fertilization on the incidence of olive fruit fly, olive leaf spot and olive anthracnose in two olive cultivars grown in rainfed conditions. Sci Hortic. 256, 108658. https://doi.org/10.1016/j.scienta.2019.108658

Rodriguez-Saona, C., Vincent, C., & Isaacs, R. 2019. Blueberry IPM: Past successes and future challenges. Annual Review of Entomology, 64(1), 95-114. https://doi.org/https://doi.org/10.1146/annurev-ento-011118-112147

Roy, S., Nuckles, E., & Archbold, D. D. 2018. Effects of Phenolic Compounds on Growth of *Colletotrichum* spp. In Vitro. Curr Microbiol. 75(5), 550-556. https://doi.org/10.1007/s00284-017-1415-7

Salotti, I., Ji, T., & Rossi, V. 2022. Temperature requirements of *Colletotrichum* spp. belonging to different clades. Front Plant Sci. 13. https://doi.org/10.3389/fpls.2022.953760

Schmid, C. J., Clarke, B. B., & Murphy, J. A. 2017. Anthracnose severity and annual bluegrass quality as influenced by nitrogen source. Crop Sci. 57(S1), S-285-S-292. https://doi.org/https://doi.org/10.2135/cropsci2016.06.0494

Sharma, S. 2020. Impacts of nitrogen on plant disease severity and plant defense mechanism. Fundam Appl Agric. 5(3), 303-314. https://doi.org/10.5455/faa.103334

Shukla, P. K., & Tarun, A. 2017. Anthracnose disease dynamics of mango orchards in relation to humid thermal ratio under subtropical climatic condition. J Agric Meteorol. 19(1), 56-61. https://doi.org/10.54386/jam.v19i1.756

Sun, Y., Wang, M., Mur, L. A. J., Shen, Q., & Guo, S. 2020. Unravelling the roles of nitrogen nutrition in plant disease defences. Int J Mol Sci. 21(2), 572. https://doi.org/10.3390/ijms21020572

Swick, M. C., Koehler, T. M., & Driks, A. 2016. Surviving Between Hosts: Sporulation and Transmission. Microbiol spectr. 4(4), 10.1128/microbiolspec.VMBF-0029-2015. https://doi.org/10.1128/microbiolspec.VMBF-0029-2015

Talhinhas, P., Gonçalves, E., Sreenivasaprasad, S., & Oliveira, H. 2015. Virulence diversity of anthracnose pathogens (*Colletotrichum acutatum* and *C. gloeosporioides* species complexes) on eight olive cultivars commonly grown in Portugal. Eur J Plant Pathol. 142(1), 73-83. https://doi.org/10.1007/s10658-014-0590-7

Uysal, A., & Kurt, Ş. 2017. Influence of inoculum density, temperature, wetness duration, and leaf age on infection and development of spinach anthracnose caused by the fungal pathogen *Colletotrichum spinaciae*. Eur J Plant Pathol. 149(4), 1041-1052. https://doi.org/10.1007/s10658-017-1249-y

Verma, N., MacDonald, L., & Punja, Z. K. 2006. Inoculum prevalence, host infection and biological control of *Colletotrichum acutatum*: causal agent of blueberry anthracnose in British Columbia. Plant Pathol. 55(3), 442-450. https://doi.org/10.1111/j.1365-3059.2006.01401.x

Walters, D. R., & Bingham, I. J. 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Ann Appl Biol, 151(3), 307-324. https://doi.org/10.1111/j.1744-7348.2007.00176.x

Wharton, P. S., & Schilder, A. C. 2008. Novel infection strategies of *Colletotrichum acutatum* on ripe blueberry fruit. Plant Pathol. 57(1), 122-134. https://doi.org/10.1111/j.1365-3059.2007.01698.x

Wichura, M. A., Koschnick, F., Jung, J., Bauer, S., & Wichura, A. 2024. Phenological growth stages of highbush blueberries (*Vaccinium* spp.): codification and description according to the BBCH scale. Botany, 102(11), 428-437. https://doi.org/10.1139/cjb-2024-0036

Xu, C. N., Zhou, Z. S., Wu, Y. X., Chi, F. M., Ji, Z. R., & Zhang, H. J. 2013. First report of stem and leaf anthracnose on blueberry caused by *Colletotrichum gloeosporioides* in China. Plant Dis. 97(6), 845-845. https://doi.org/10.1094/pdis-11-12-1056-pdn

INDICE DE TABLAS

Table 1. Nitrogen fertilization treatments and macro and micronutrient solution applied during the experiment

Fertilizer	Rate (g plant^{-1} d^{-1})	Total rate of applied nutrients during the growing season (g plant^{-1} season^{-1})					
Ammonium sulfate treatment		N	P2O5	K2O	CaO	MgO	S
N0 (control)	0	0					0
N1x	0.16	19.8					22.7
N2x	0.32	39.7					45.3
N3x	0.48	59.9					68
Macro and micronutrient solution							
Monoammonium phosphate	0.052	0.8	1.8				
Potassium sulfate	0.216			11.7			5
Calcium nitrate	0.631	12.6			15.1		
Magnesium sulfate	0.076					1.1	2.1
Mix of macronutrient [a]	0.539						1.9

[a] Fetrilon Combi 2®: B 1.5%, Cu 0.6%, Fe 4.0%, Mn 3.0%, Mo 0.05%, Zn 4.0%.

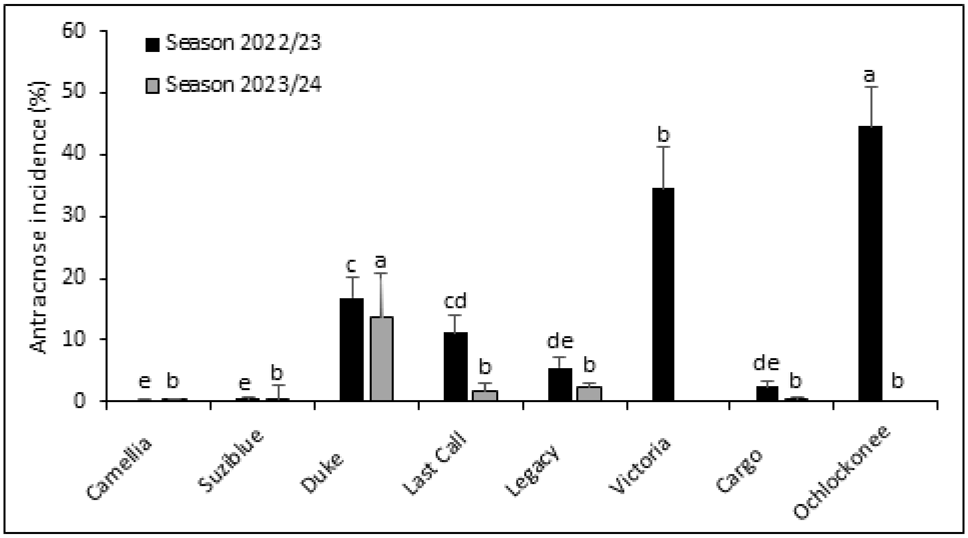
Table 2. Significance analysis of the incidence and severity of anthracnose caused by *Colletotrichum fioriniae* in fruits and proportion of necrotic leaf area (NLA), in different blueberry cultivars fertilized with increasing rates of nitrogen during the 2022/23 and 2023/24 seasons.

Variation source	Incidence *C. fioriniae*	Severity *C. fioriniae*	NLA *C. fioriniae*	NLA *Botrytis* sp.
Season 2022-2023				
Cultivar (C)	**	**	**	**
N fertilization level (N)	ns	ns	ns	*
Interaction C*N	**	**	**	**
Season 2023-2024				
Cultivar (C)	**	**	**	**
N fertilization level (N)	ns	ns	ns	**
Interaction C*N	ns	*	**	**

ns: no significant; *significant ($P < 0.05$) and ** significant ($P < 0.01$)

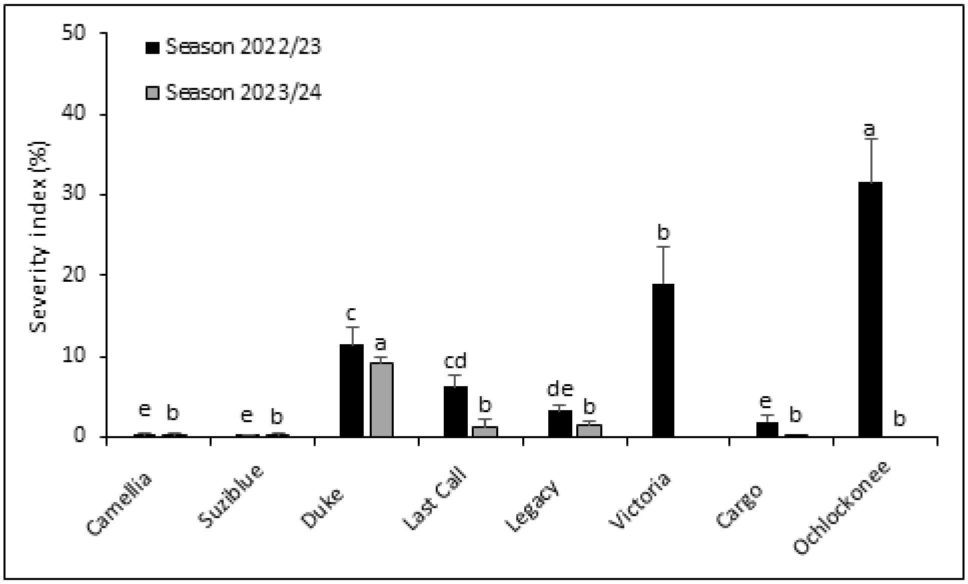
INDICE DE FIGURAS

Figure 1. Incidence of anthracnose in fruits of different blueberry varieties inoculated with the pathogen *Colletotrichium fioriniae* during two experimental seasons.



Data correspond to the average between the different nitrogen fertilization treatments per cultivar (n=12). Different letters above bars of the same color indicate significant differences between cultivars in bars of the same color (season). The error bars correspond to the standard error. Cultivar Victoria was not analyzed during the 2023/24 season because it presented fruiting problems.

Figure 2. Anthracnose severity index in fruits of blueberry plants of different varieties inoculated with the pathogen *Colletotrichum fioriniae* during two experimental seasons.



Data correspond to the average between the different nitrogen fertilization treatments per cultivar (n=12). Different letters above bars of the same color indicate significant differences between cultivars (season). The error bars correspond to the standard error. ‘Victoria’ was not analyzed during the 2023/24 season because it presented fruiting problems.

Figure 3. Necrotic lesions with the presence of acervuli and masses of conidia on the surface of blueberry leaves inoculated with *Colletotrichum* fioriniae in a humid chamber under controlled conditions.

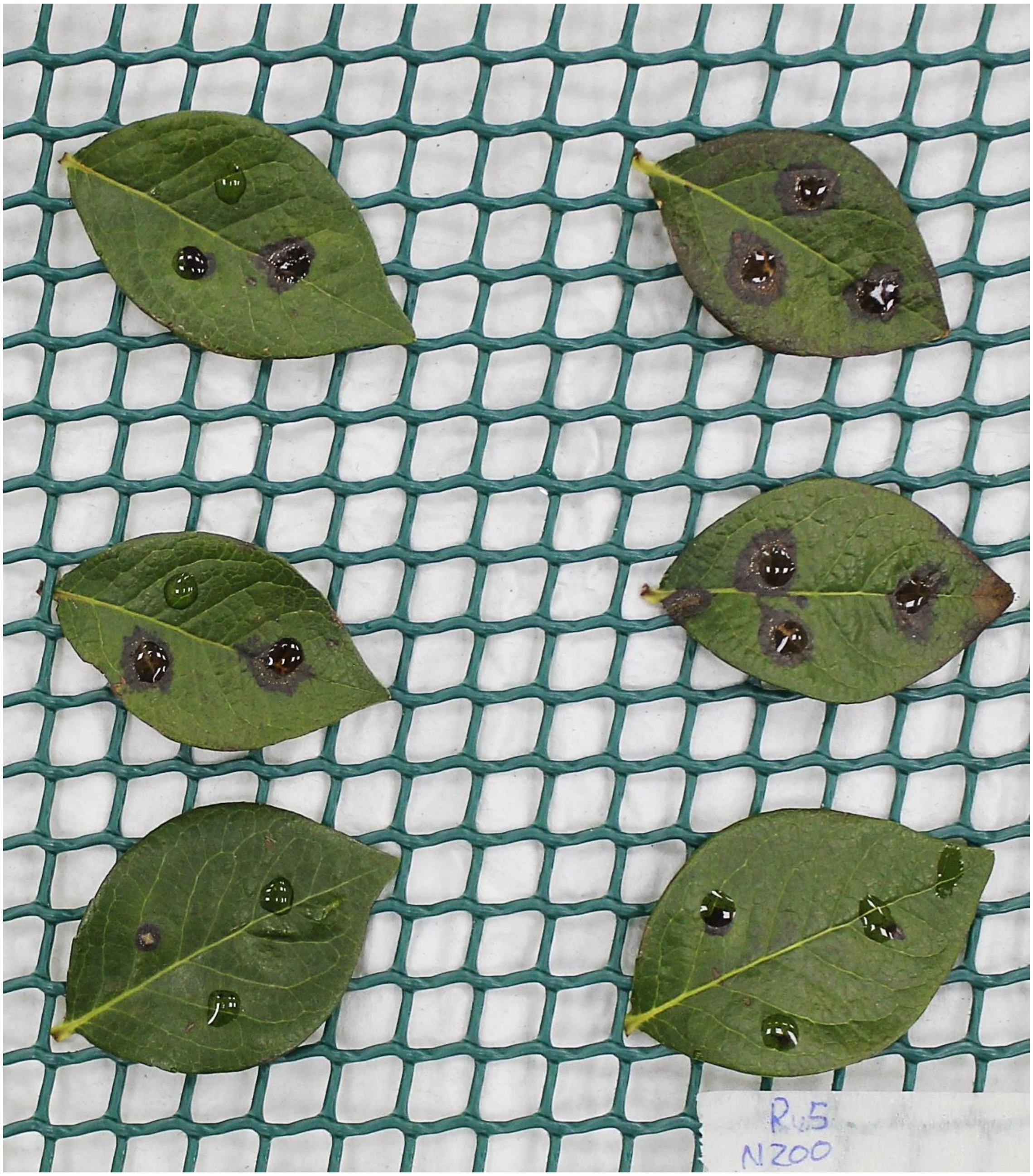
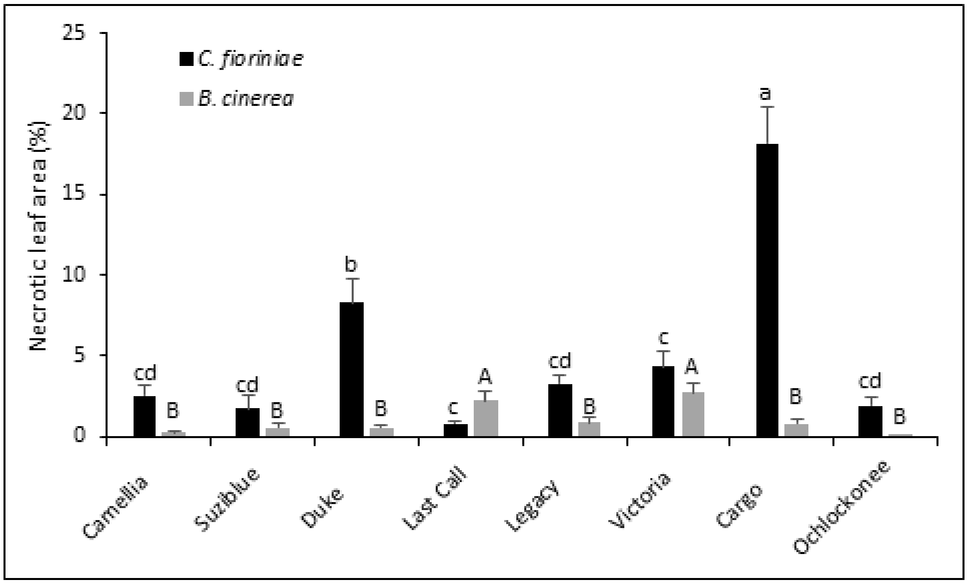
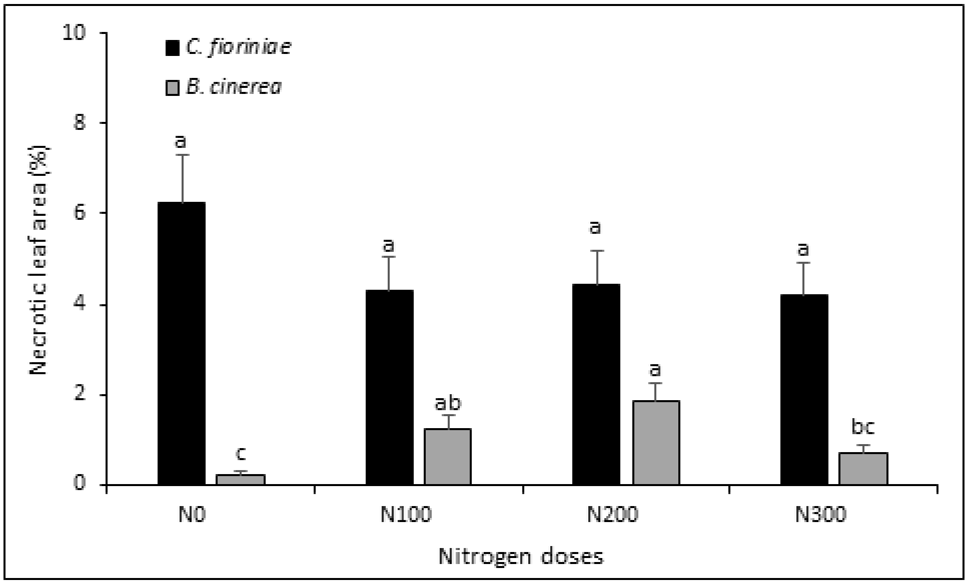


Figure 4. Proportion of necrotic leaf area (NLA) of blueberries of different varieties inoculated with the pathogen *Colletotrichum fioriniae* and *Botrytis cinerea* evaluated during two experimental seasons.



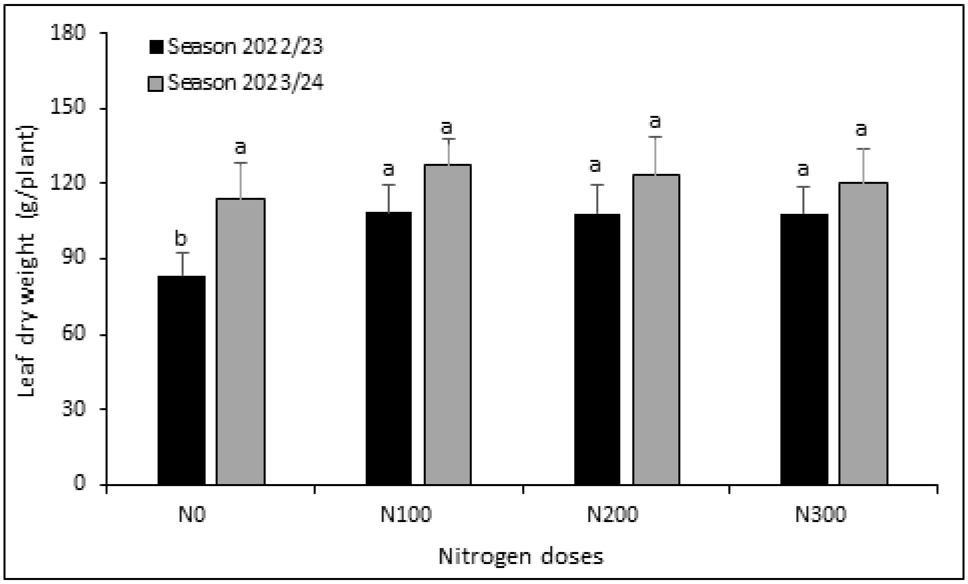
Data correspond to the average between the different nitrogen fertilization treatments per cultivar (n=12). Different letters above bars of the same color indicate significant differences between cultivars, with respect to *C. fioriniae* or *B.cinerea* according to the least significant difference (LSD) test ($p < 0.05$). Error bars correspond to the standard error.

Figure 5. Proportion of necrotic leaf area (NLA) of blueberries fertilized with increasing doses of nitrogen (N0 = 0, N1x = 19.8, N2x = 39.7 and N3x = 59.9 g plant season^{-1}) and inoculated with the pathogen *Colletotrichum fioriniae* and *Botrytis cinerea* evaluated during two experimental seasons.



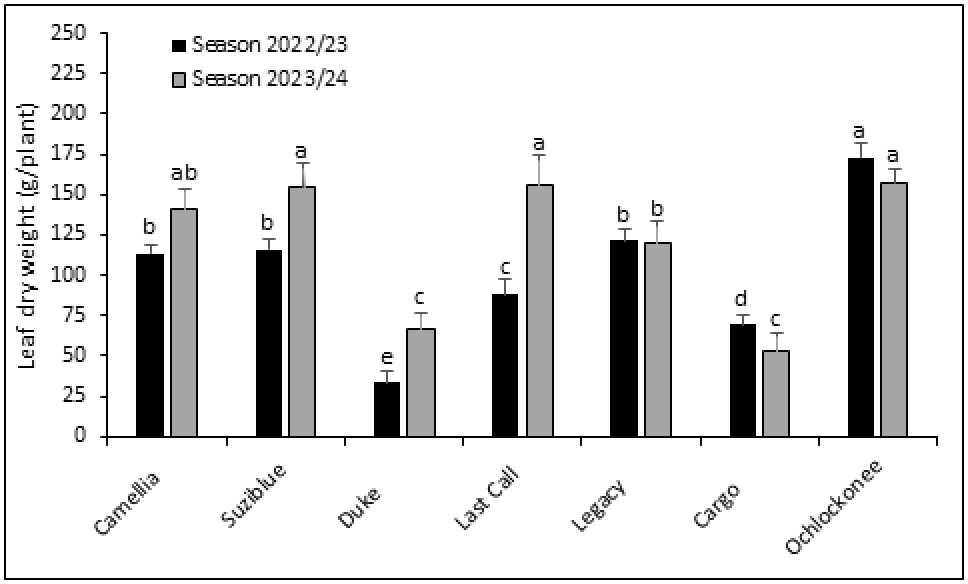
Data correspond to the average between the different cultivars for each nitrogen dose (n=24). Different letters above bars of the same color indicate significant differences between cultivars, with respect to *C. fioriniae* or *B.cinerea* according to the least significant difference (LSD) test ($p < 0.05$). Error bars correspond to the standard.

Figure 6. Average dry biomass of leaves of different blueberry cultivars according to their fertilization rate (N0 = 0, N1x = 19.8, N2x = 39.7 and N3x = 59.9 g plant season^{-1}), during the 2022/23 and 2023/24 seasons.



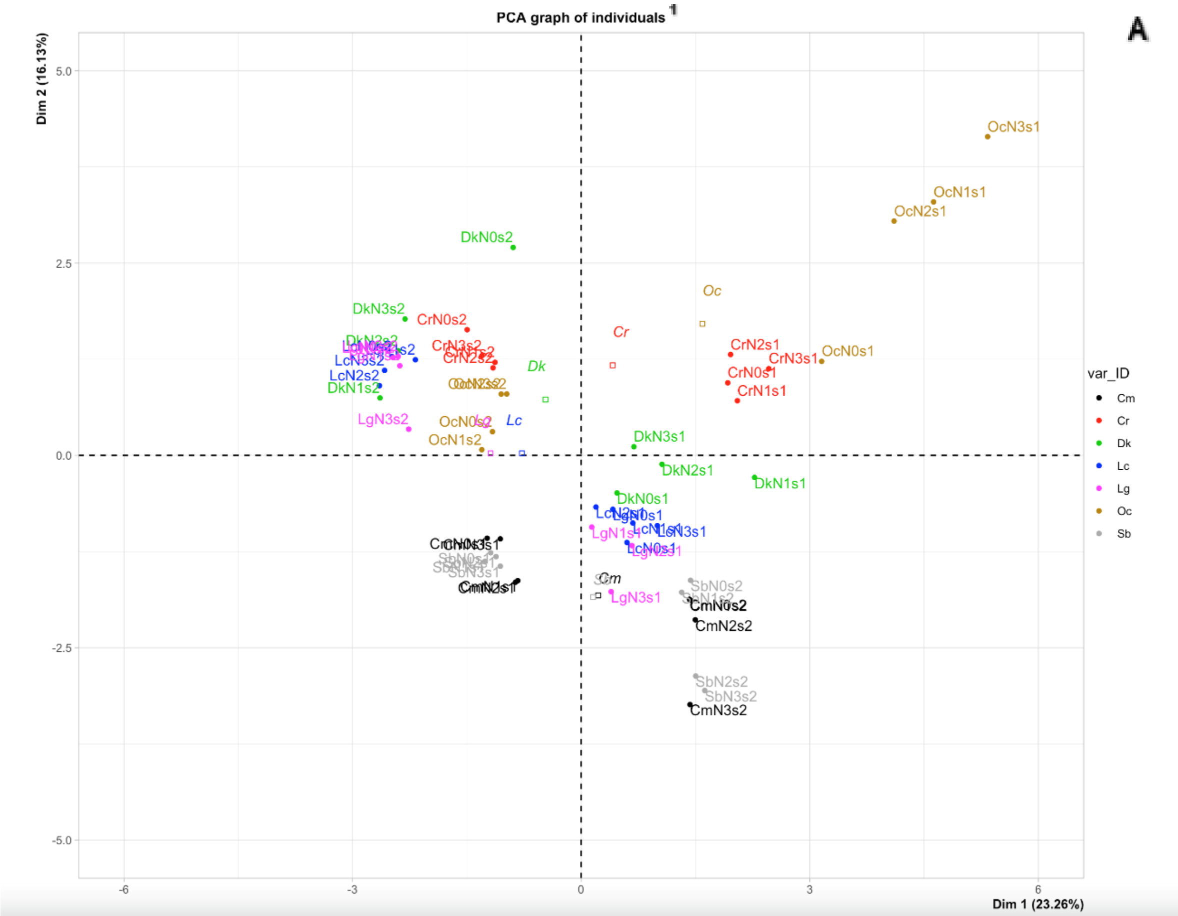
Data correspond to the average between the different cultivars for each nitrogen dose (n=24). Different letters above bars of the same color indicate significant differences between cultivars, with respect to *C. fioriniae* or *B.cinerea* according to the least significant difference (LSD) test ($p < 0.05$). Error bars correspond to the standard. 'Victoria' was not analyzed because it presented defoliation.

Figure 7. Dry biomass of leaves of different blueberry cultivars fertilized with increasing rates of nitrogen (N0 = 0, N1x = 19.8, N2x = 39.7 and N3x = 59.9 g plant season-1), during the 2022/23 and 2023 / 24 seasons.

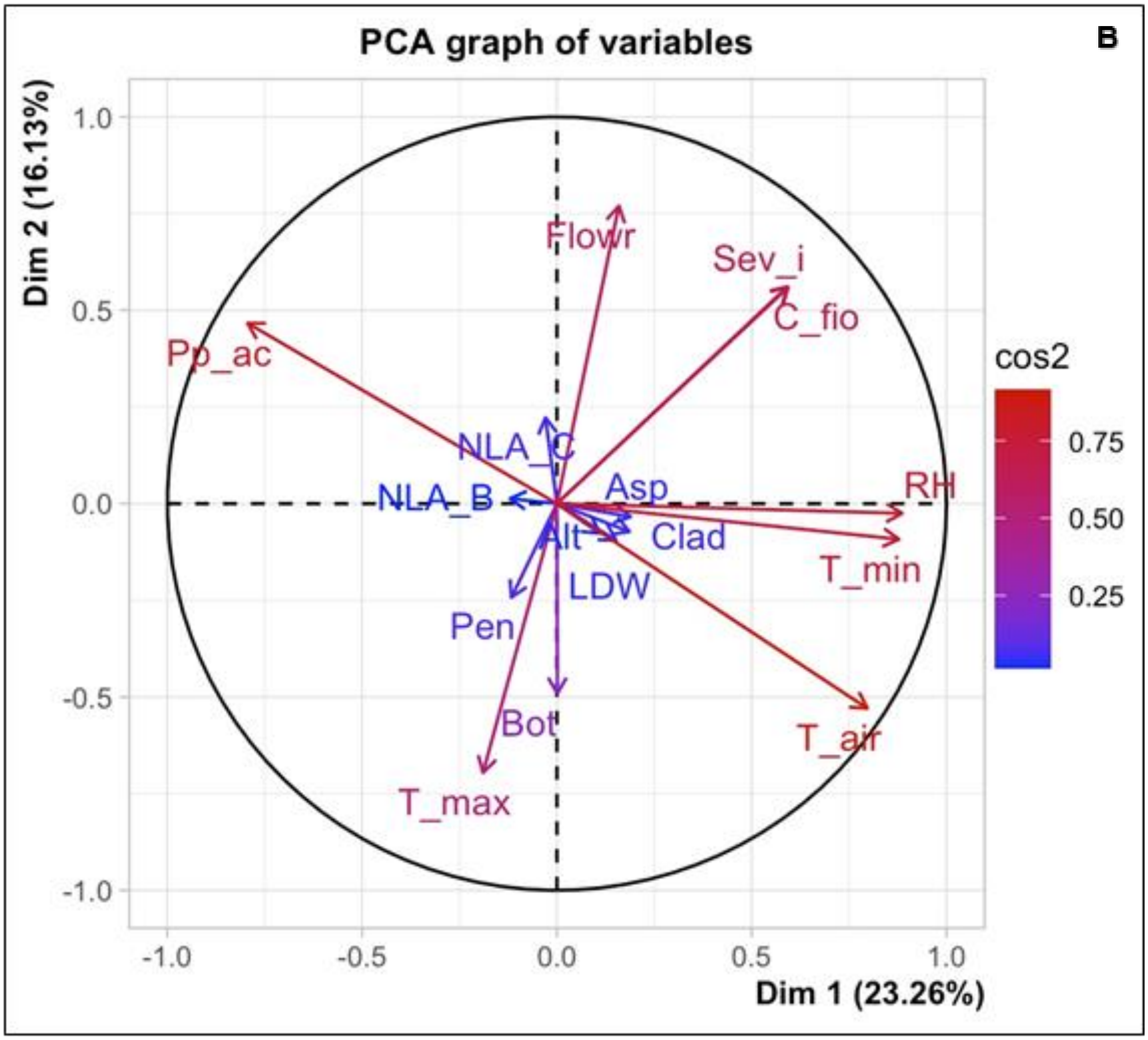


Data correspond to the average between the different nitrogen fertilization treatments per cultivar (n=12). Different letters above bars of the same color indicate significant differences between cultivars, with respect to *C. fioriniae* or *B.cinerea* according to the least significant difference (LSD) test ($p < 0.05$). Error bars correspond to the standard error. 'Victoria' was not analyzed because it presented defoliation.

Figure 8. Principal component analysis (A) and variable graph (B) of different blueberry cultivars, fertilized with increasing doses of nitrogen (N0 = 0, N1x = 19.8, N2x = 39.7 and N3x = 59.9 g plant season^{-1}), during two evaluation seasons (S1 2022/23 and S2 2023/24).



[1]Individuals code: Cm: Camellia; Cr: Cargo; Dk: Duke; Lg: Legacy; Lc: Last Call; Sb: Suziblue; Oc: Ocholocknee.


PCA graph of variables
B
Dim 2 (16.13%)
Dim 1 (23.26%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Flowr
Sev_i
C_fio
Pp_ac
NLA_C
NLA_B
Asp
RH
Alt
Clad
T_min
LDW
Pen
Bot
T_air
T_max
cos2
0.75
0.50
0.25

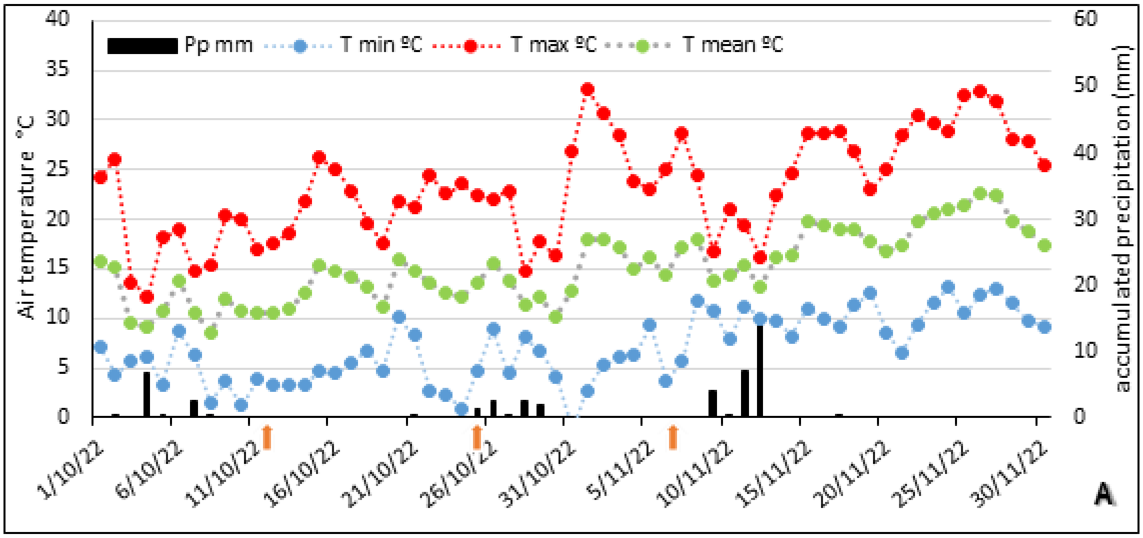
SUPPLEMENTARY MATERIAL

Table 1. Rating scale of anthracnose disease severity in blueberry fruits.

Score	Disease severity and symptoms description
0	Healthy fruit, no symptoms (0%)
1	Fruits show discoloration and sparse acervuli with orange conidial masses covering between 1% to 5% of the fruit area.
2	Fruits with discoloration and/or sunken areas with acervuli containing orange conidial masses covering 5% to 25% of the fruit area.
3	Fruits exhibit sunken areas with extensive acervuli and orange conidial masses covering 25% to 50% of the fruit area.
4	Fruits show sunken areas with extensive acervuli and orange conidial masses covering 50% to 75% of the fruit area.
5	Fruits partially or fully colonized by the fungus, with acervuli and orange conidial masses covering 75% to 100% of the fruit area. Occasionally, the fruits collapse.

* adaptation of Moral and Trapero (2009)

Figure 1. Environmental conditions and inoculation dates during 2022 (A) and during 2023 (B). Inoculation dates are marked by an orange arrow on the time axis.



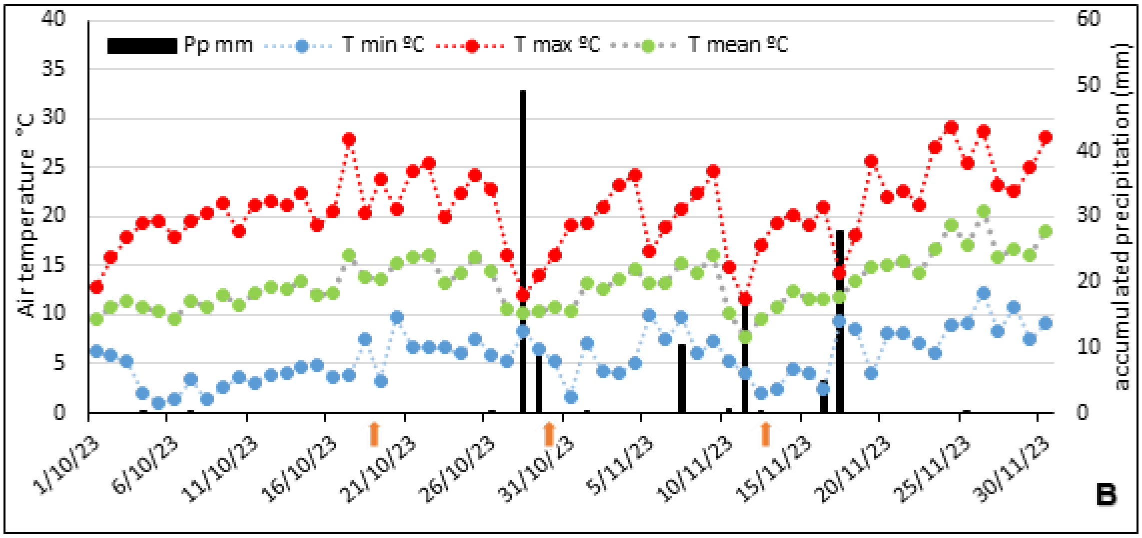


Figure 2. Anthracnose severity scale in postharvest blueberry fruits

CONCLUSIONES GENERALES

Los resultados obtenidos en esta investigación indican que la presencia de inóculo y las condiciones ambientales durante la floración y el desarrollo del fruto son determinantes en la incidencia y severidad de antracnosis causada por *Colletotrichum fioriniae*, en cultivares de arándano de las especies *Vaccinum corymbosum* híbrido, *V. corymbosum* y *V. virguinatum*, con diferentes periodos de floración. Cultivares como ‘Camellia’ y ‘Suziblue’, caracterizados por un menor requerimiento de frío y floración temprana, mostraron una menor susceptibilidad a antracnosis en ambas temporadas de evaluación. Por el contrario, los cultivares de cosecha media y tardía, como 'Duke', 'Last Call', 'Victoria' y 'Ochlockonee', mostraron una mayor susceptibilidad a la enfermedad cuando las condiciones ambientales son favorables para la infección por el patógeno. Además, la fertilización con nitrógeno no tuvo impacto significativo en la incidencia o severidad de *C. fioriniae* en los cultivares evaluados. A su vez, tanto *C. fioriniae* como *Botrytis cinerea* fueron capaces colonizar las hojas de arándano y provocar manchas necróticas, pero sólo *B. cinerea* mostró un aumento de la necrosis foliar asociado a un incremento de la fertilización nitrogenada.