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A Rapid and Cost-Effective Flow Cytometry Method for Detecting Air Inoculum of Botrytis cinerea and Aspergillus carbonarius in Vineyards

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Abstract

Bunch rot, caused by phytopathogenic fungi, such as *Aspergillus carbonarius* and *Botrytis cinerea*, is one of the main causes of damage in both table grape and wine grape production, resulting in substantial economic losses and food safety risks due to human exposure to mycotoxins. The aim of this work was to develop a morphology-based flow cytometry diagnostic tool capable of discriminating the airborne conidia of the target species and enabling timely intervention. To achieve this goal, conidial suspensions of *A. carbonarius* and *B. cinerea* were first characterized individually, together with those of the fungal species commonly found simultaneously on grapes in the vineyard and in postharvest storage environments. Once the conidial populations had been characterized, gates constructed from mixed conidial suspensions and from samples collected using spore traps were used to validate the specificity of the method in the simultaneous presence of multiple fungal species. The result was a rapid, cost-effective, and environmentally friendly method for analyzing collected air samples. Results were also validated using biological and molecular methods. This technique represents a promising strategy for the characterization and discrimination of airborne spores and may also find useful applications in more complex matrices such as washing water.

1. Background

Bunch rots represent one of the major threats to grapevine production, negatively affecting fruit quality and safety and causing significant economic losses (1).

Among grape bunch rots, grey mold, caused by *Botrytis cinerea*, is one of the most economically important grapevine diseases worldwide (2–4). On table grapes, *B. cinerea* infections are particularly relevant because they compromise grape quality and postharvest shelf life, leading to significant commercial losses (5). The epidemiology of *B. cinerea* is strongly influenced by environmental conditions and is closely linked to the production and dispersal of conidia, which represent the primary source of inoculum. Therefore, monitoring airborne propagules of *B. cinerea* is a valuable tool for assessing infection risk and supporting disease forecasting models (6, 7).

Aspergillus carbonarius is frequently associated with secondary bunch rot, particularly on winegrapes in warm viticultural areas (8–10). In grapes and grape-derived products, *A. carbonarius* is widely recognized as the main source of ochratoxin A (OTA) contamination (10–12). This contamination represents a major food safety concern because of the nephrotoxic and potentially carcinogenic effects of the mycotoxin. Similarly to *B. cinerea*, airborne conidia of *A. carbonarius* play a crucial role in pathogen dissemination and berry colonization, particularly during the late ripening stages. A positive correlation between berry contamination and subsequent OTA accumulation has been reported (8, 13, 14). Aerobiological monitoring using spore traps is increasingly used to assess the dynamics of fungal conidia in vineyard environments. However, the identification of trapped spores is still predominantly based on microscopy, culture-dependent methods or molecular techniques. Although effective, these approaches are time-consuming and require highly specialized expertise. In the context of integrated pest management, there is an increasing demand for rapid, reliable, and low-cost diagnostic tools. Therefore, innovative analytical techniques reducing analysis time and operational costs are highly desirable.

Flow cytometry is a high-throughput technology that enables rapid analysis of particles and cells suspended in a fluid stream by simultaneously measuring light scattering and fluorescence signals (16). Based on the optical interrogation of individual particles flowing in a laminar stream, flow cytometry allows multiparametric analysis based on size, internal complexity, and optical or biochemical properties, especially when combined with fluorescent stains or intrinsic autofluorescence (17). This analytical technique has been extensively applied in biology, medical diagnosis, environmental microbiology, food safety and chemistry (18–20). In recent years, phytopathological applications of flow cytometry have focused mainly on the detection of bacterial pathogens (21, 22), whereas reports on fungal

pathogen detection remain limited (23–27). In aerobiological studies, flow cytometry offers significant advantages over conventional methods, including high analytical speed, minimal sample preparation and the absence of harmful and/or polluting reagents (28, 29). These features make it particularly suitable for the rapid analysis of airborne samples, and for early pathogen detection, which is essential for timely disease prevention and management.

Despite the growing interest in rapid diagnostic technologies, the application of flow cytometry to aerobiological samples from vineyards remains largely unexplored. To date, most studies have focused on pure cultures or laboratory-based detection of microorganisms, whereas validation for the direct analysis of airborne fungal propagules collected under field conditions is still lacking.

This study aimed to develop and validate a rapid and low-cost flow-cytometric diagnostic method for the detection of *B. cinerea* and *A. carbonarius* in airborne samples. The method was optimized to specifically and sensitively discriminate *A. carbonarius* and *B. cinerea* conidia population from environmental contaminants and spores of non-targeted species.

2. Methods

2.1 Strains and growing conditions

The fungal target strains, used in this work, (Table 1) were maintained as conidial suspension containing 20% glycerol at − 80°C in the culture collection of the Department of Soil, Plant and Food Sciences of the University of Bari Aldo Moro (Italy). Strains were revitalized and routinely cultured at 25°C in the dark on Potato Dextrose Agar (PDA: infusion of 200 g peeled and sliced potatoes kept at 60°C for 1 h, 20 g of dextrose, 20 g of agar (European bacteriological agar, LLG-labware, Meckenheim, Germany), per liter, adjusted at pH 6.5).

Table 1
Fungal strains used in this study

Species (strain)	Substrate of isolation	Locality	Deposition year
Alternaria alternata (Aa1)*	Cherry	Turi (Ba, Italy)	-
Aspergillus carbonarius (AC49; CBS 144853)	Wine grapes (‘Negroamaro’)	Leverano (Le, Italy)	2000
Aspergillus niger (An1)*	Wine grapes (‘Primitivo’)	Sava (Ta, Italy)	2000
Botrytis cinerea (SAS56; CBS 145097)	Obtained from sexual cross, in laboratory conditions	Bari (Italy)-	1987
*Cladosporium sp.**	Pomegranate	Rutigliano (Ba, Italy)	2018
*Penicillium expansum**	**	**	2022
** fungal strain used as control;*			

***data unknown, strain kindly supplied by Prof. Antonio Ippolito.*

2.2 Spore suspensions preparation

Conidia of *B. cinerea* were obtained as described by Faretra et al. (30); all other isolates were grown on PDA for 7 days at 25°C in the dark. Fresh conidial suspensions were prepared by gently scraping the colony surface with a sterile spatula and resuspending the spores in sterile distilled water. Suspensions were filtered through sterile Miracloth to

remove mycelial debris. Spore suspensions were quantified by using a hemocytometer and adjusted at 1×10^6 conidia×mL^{-1}. To uniquely identify the populations fluorometrically, conidia were stained with 20 µM Calcofluor White Stain (Sigma-Aldrich, St. Louis, MO, USA), washed twice with sterile distilled water and finally resuspended in 1 mL of sterile distilled water prior to analysis.

2.3 Airborne samples collection for method validation

Air-borne inoculum samples were collected using an air sampler consisting of an electric pump operating at an airflow rate of 300 L×•min^{-1}, with an exhausting pressure of 4.5 kPa and maximum aerating pressure of 4 kPa. The system was connected to a filter holder equipped with 0.2 µm nylon membrane filters (MilliporeSigma, Burlington, MA, USA). Following sampling, spores retained on the filters were resuspended in sterile distilled water and immediately analyzed. For validation on artificially inoculated samples, five berries of 'Sugar Crisp™' were inoculated with 10 µL of conidial suspension (1×10^5 conidia×mL^{-1}) of each target species. Berries were incubated in a humid chamber at 25°C for five days, allowing fungal growth and sporulation. Finally, the method was further validated on naturally contaminated grape samples of the table grape varieties Allison and Autumn crisp, harvested from two different vineyards located in the area of Sammichele di Bari (Ba, Italy; 40°58'46.1"N 17°00'14.0"E and 40°54'35.1"N 16°54'17.1"E).

2.4 Flow cytometry analysis

The analyses were performed using a BD LSRFortessa X-20 Cell Analyzer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), equipped with configurable polygon-shaped optical pathways that maximize signal detection through signal reflection. Forward scatter (FSC-A) and side scatter (SSC-A) were acquired using a 488 nm laser (laser power 50 mW) as light source. Both OTA and Calcofluor White Stain fluorescence signal were acquired by exciting the molecule with a 405 nm laser (laser power 50 mW), and the emission was detected through a 450 ± 25 nm bandpass filter. Ten-thousand events were acquired for each sample using a flow rate of 12 µL×min^{-1}. Data were analyzed using BD FACSDiva™ software.

2.5 Flow cytometry results validation through biological and molecular techniques

To validate flow cytometry results, viable fungal propagules were quantified biologically. An aliquot of 100 µL of each sample was serially diluted (10-fold dilutions) in sterile distilled water and plated in triplicate on Petri dishes with Malt Extract Agar [MEA, 20 g of Malt Extract (Oxoid, Wesel, Germany), and 20 g of agar (European bacteriological agar, LLG-labware) per liter]. Plates were incubated at 25°C in the dark. After three days, the developed colonies were counted and morphologically identified. To discriminate between *A. niger* and *A. carbonarius*, a species-specific qPCR protocol (13) was also applied. Briefly, 2 mL of each sample were centrifuged at 17.0 × g for 30 minutes. Pellets were collected and genomic DNA was extracted using a modified CTAB method (31). The resuspended DNA of each sample was then analyzed by qPCR in a CFX96™ Real-Time PCR Detection System Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA).ù

2.6 Statistical analysis

The correlation between the data obtained by flow cytometry and those obtained by biological and molecular assays were calculated using Microsoft Excel software ver. 365.

3. Results

3.1 Spore suspension analysis

Flow cytometry analysis enabled the unequivocally discrimination of fungal spore populations in aqueous samples. Conidial suspensions obtained from pure cultures were stained with Calcofluor White, allowing reliable identification of fluorescent spore populations detected in the 450 ± 25 nm emission band, consistent with dye binding to chitin in the fungal cell wall. Distinct spore population of *A. alternata*, *A. carbonarius*, *A. niger*, *B. cinerea*, *Cladosporium sp.*, and *Penicillium sp.* were identified by combining fluorophore labelling (which allowed fluorescent events to be selected by applying a minimum threshold) with the dimensional (FSC-A) and the structural (SSC-A) scattering characteristics of the corpuscles. Based on the flow cytometry profiles obtained from pure-culture suspensions, species-specific gates were established using FSC-A and SSC-A parameters. Gates were defined to include at least 60% of total events attributable to each target population (Fig. 1), allowing exclusion of events with morphological characteristics more distant from the core of each population in the dot plot. The combined use of fluorescence and scatter parameters provided a reliable and reproducible identification and characterization of specie-specific spore populations for *A. carbonarius*, *A. niger, B. cinerea*, *Cladosporium sp.*, and *Penicillium sp.* (Fig. 2). However, *A. alternata* could not be accurately discriminated because its spore population overlapped with non-specific background events within the corresponding dot plot region (Fig. 3).

To assess potential of false-positive events, each species-specific gate was overlaid onto the total event populations of all other. This approach allowed the determination of the misclassification rate associated with the adopted gating strategy. The resulting misclassification matrix (Fig. 3) quantified the percentage of off-target events falling within each gate. Overall, false-positive rates were generally low (< 5%) for most species combinations. *B. cinerea* showed near-complete separation from other species, with misclassification value approaching zero and not exceeding 2% for *A. carbonarius*. In contrast, substantial overlap was observed between *Cladosporium sp.* and *P. expansum*, with reciprocal misclassification rates reaching 41.5% and 43%, respectively, thereby preventing reliable discrimination between these two taxa.

Table 2

		Species-specific gate applied					
		Alternaria alternata	*Aspergillus carbonarius*	*Aspergillus niger*	*Botrytis cinerea*	*Cladosporium sp.*	*Penicillium expansum*
Individual species	*Alternaria alternata*		0.0 ± 0.0	2.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Aspergillus carbonarius	0.0 ± 0.0		4.2 ± 0.2	1.9 ± 0.0	6.3 ± 0.2	2.7 ± 0.2
	Aspergillus niger	0.0 ± 0.0	7.1 ± 0.2		0.0 ± 0.0	3.6 ± 0.1	5.2 ± 0.3
	Botrytis cinerea	0.0 ± 0.0	3.8 ± 0.1	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0
	Cladosporium sp.	0.0 ± 0.0	12.3 ± 0.2	5.2 ± 0.2	0.1 ± 0.0		41.5 ± 1.5
	Penicillium expansum	0.0 ± 0.0	0.8 ± 0.1	1.3 ± 0.0	0.7 ± 0.0	43 ± 1.5	

Percentage (%) of events classified as positive within each species-specific gate (columns) when applied to the total event population of the corresponding fungal species (rows). This matrix represents the degree of overlap and potential false-positive rates among different fungal spore populations based on the established gating strategy.

To evaluate gating performance under complex conditions, species-specific gates were applied to a mixed spore suspension containing equal proportions (1:1 ratio) of all tested species (*A. alternaria*, *A. carbonarius*, *A. niger*, *B. cinerea*, *Cladosporium sp.*, and *P. expansum*). The predefined gating strategy successfully discriminated *A. carbonarius*, *A. niger* and *B. cinerea* populations. As observed in pure suspension and as expected, *Cladosporium sp.* and *P. expansum* populations remained largely overlapping and could not be reliably distinguished. Furthermore, the gate corresponding to *A. alternata* was populated predominantly by non-specific events, confirming the limitation observed previously and preventing reliable gate application. The distribution of events across the discriminable species-specific gates allowed detection of the spores of each target species, supporting the robustness of the gating strategy in mixed samples (Fig. 4).

3.2 Validation on airborne samples

To preliminary assess the applicability of method under field-like conditions, airborne spores were collected from artificially inoculated berries. Flow cytometry analysis allowed the correct discrimination of the target populations, with scatter profiles consistent with those previously established using pure conidial suspensions, thereby enabling a reliable attribution of the events to the corresponding fungal species.

The method was subsequently validated on five naturally contaminated air-borne samples (Table 3).

Table 3

Sample ID	***Percentage of recorded events in each species-specific gate***		
	Aspergillus carbonarius	***Aspergillus niger***	***Botrytis cinerea***
1	8.6	1.2	4.0
2	11.0	2.8	23.0
3	2.1	20.6	24.3
4	2.5	24.6	18.0
5	1.6	10.3	33.9

Flow cytometry classification of spores detected in five samples collected from naturally contaminated grapes. Results are expressed as percentage of spores counted for each specific gate out of a total of 10,000 events.

In all samples, the most frequently detected events were attributable to the combined *Penicillium*/*Cladosporium* gate, except for sample n. 2, in which *B. cinerea* was consistently detected at appreciable levels. A significant proportion of events attributable to *A. carbonarius* was observed only in sample 2. *A. niger* was scarcely detected in samples 1 and 2, but it was more abundant in samples 3, 4 and 5, where the associated gate was densely populated.

3.3 Biological and molecular characterization of natural airborne samples

Flow cytometry results were validated by culture-based quantification. *Aspergillus* spp., *B. cinerea* and *Penicillium* spp. were detected in all samples, whereas neither *A. alternata* nor *Cladosporium* spp. were recovered by plating. *Penicillium* spp. represented the prevalent culturable population in the samples 1, 3 and 4, reaching concentrations up to 115.0×10³ CFU/mL. *Aspergillus* spp. and *B. cinerea* were consistently detected, with concentrations ranging from 5.0 to 11.0×10³ CFU/mL and 3.7 to 31.5×10³ CFU/mL, respectively (Table 4).

Table 4

Sample ID	*Morphology (CFU×10^3/mL)*				
	Alternaria alternata	***Aspergillus* section Nigri***	***Botrytis cinerea***	***Cladosporium spp.***	***Penicillium spp.***
1	0.0	8.0	7.5	0.0	100.0
2	0.0	11.0	31.5	0.0	12.0
3	0.0	5.0	30.5	0.0	53.5
4	0.0	5.0	18.0	0.0	115.0
5	0.0	7.0	3.7	0.0	20.0

** In the table*, Aspergillus *section Nigri were grouped due to their morphological similarity.*

Colony-Forming Units (CFU) per milliliter of fungal morphotypes detected in five samples collected from naturally contaminated grapes.

Samples were taken from colonies identified as belonging to the genus *Aspergillus* and observations were made under a stereomicroscope. A strong positive correlation was observed between the presence of flow cytometry events attributable to *A. carbonarius* and *B. cinerea* populations and their corresponding colony-forming units, withs R^2 values of 0.89 and 0.96, respectively. In contrast, the correlation between the estimated number of *A. niger* colonies and the events recorded by flow cytometry was very low. Furthermore, the presence of *A. carbonarius* in the five naturally infected samples was also validated by qPCR. Quantitative results showed high correlation between qPCR and culture-based stereoscopic quantification (R^2= 0.85), as well as between qPCR and flow cytometry events counts (R^2= 0.75).

4. Discussions

In viticulture, the early detection of airborne inoculum of *B. cinerea* and *A. carbonarius* is essential for disease management and for reducing postharvest losses and mycotoxin contamination, particularly ochratoxin A (OTA) accumulation in grapes and derived products (5, 10–12, 15). Despite the availability of multiple diagnostic approaches, reliable high-throughput monitoring of air propagules in field-relevant conditions remains challenging.

Conventional aerobiological monitoring relies primarily on microscopy-based spore identification and volumetric trapping systems. Although widely used, these approaches are limited by operator dependency, low analytical throughput, and reduced sensitivity toward small, hyaline, or morphologically similar conidia such as those of *B. cinerea* and related taxa (32, 33). Culture-based methods, while still considered a reference standard for viability assessment, are inherently biased by selective growth conditions and fail to detect non-culturable or stressed propagules.

Molecular diagnostic tools, including PCR, qPCR, and LAMP-based assays, have significantly improved detection sensitivity and specificity for both *B. cinerea* and *A. carbonarius* in grapevine berries and plant tissues as well as in environmental samples as airborne spore and bioaerosol matrices (34–37). More recently, gene-targeted assays focusing on functional elements of ochratoxin A biosynthesis pathways, such as the *AcOTApks* gene, have enabled improved discrimination of toxigenic strains and more accurate quantification of pathogenic potential (13). However, these methods still require nucleic acid extraction, laboratory infrastructure, and relatively long processing times, limiting their suitability for high-frequency or near-real-time monitoring applications.

In this context, morphology-based flow cytometry provides an alternative label-free approach for rapid spore detection, based on intrinsic optical properties (forward and side scatter). Previous studies have demonstrated the feasibility of flow cytometric detection of airborne fungal spores and plant pathogenic propagules, including oomycete sporangia (*Phytophthora infestans*), bacterial plant pathogens (*Xanthomonas campestris*), and complex airborne fungal communities, as well as its application in environmental aerobiology and plant disease diagnostics (21, 24, 25, 29, 38). The present study extends these approaches by applying species-specific gating strategies to vineyard-relevant fungal pathogens.

The results demonstrated that *B. cinerea* and *A. carbonarius* can be reliably discriminated using scatter-based cytometric signatures, with low misclassification rates (< 2% for *B. cinerea* and generally < 5% for *A. carbonarius*) and strong agreement with culture-based quantification (R^2 = 0.96 and 0.89 for *B. cinerea* and *A. carbonarius*, respectively). The additional validation through qPCR further supported the reliability of the approach, with a substantial correlation (R^2 = 0.75) between molecular and cytometric data for *A. carbonarius*. Overall, these results highlight that differences in spore morphology and internal complexity can be effectively exploited to achieve robust discrimination of taxonomically distinct airborne pathogens under controlled gating conditions. However, performance varied across taxa. Significant overlap was observed between *Cladosporium* sp. and *P. expansum*, with reciprocal misclassification rates approaching 40%, highlighting the limitations of scatter-only approaches when applied to morphologically similar or environmentally variable fungal groups. Similarly, *A. alternata* could not be reliably discriminated from background particulate matter, likely due to the high variability of its scatter-based cytometric signature and its overlap with heterogeneous airborne biological and non-biological particles, as previously observed in aerobiological flow cytometry studies (27). The discrepancy observed for *Aspergillus niger* between pure cultures and environmental samples further suggests that natural *Aspergillus* section Nigri diversity and co-occurring morphotypes can reduce classification robustness. This is consistent with previous studies on vineyard fungal communities showing that *Aspergillus* populations are highly heterogeneous and strongly influenced by spatial variability in soil and environmental reservoirs, requiring highly sensitive molecular approaches such as droplet digital PCR for accurate quantification (39).

These findings indicate that morphology-based flow cytometry is most effective as a targeted detection tool for specific airborne pathogens, rather than as a comprehensive fungal community profiling method. While it performs well for clearly distinct taxa such as *B. cinerea*, its discriminatory power decreases for closely related or morphologically overlapping species, where additional molecular or fluorescence-based markers may be required.

Despite these limitations, the method offers several operational advantages. Compared to microscopy, it reduces operator bias and significantly increases throughput. Compared to molecular techniques, it eliminates nucleic acid extraction and hazardous reagents, enabling faster and more streamlined analysis workflows. Within the current diagnostic landscape, flow cytometry occupies an intermediate position between high-specificity molecular assays and low-throughput microscopic observation, combining rapid analysis with moderate taxonomic resolution.

From an applied perspective, rapid detection of airborne inoculum of *B. cinerea* and *A. carbonarius* may enhance disease forecasting and support data-driven vineyard management strategies. Early quantification of spore pressure has the potential to enable timely, targeted interventions, improving the efficiency of fungicide applications while reducing overall chemical inputs, in line with aerobiological and epidemiological studies highlighting the importance of airborne inoculum in disease development(6, 15). Moreover, improved monitoring of *A. carbonarius* airborne inoculum may contribute to reducing the risk of OTA contamination in grapes and wine, thereby addressing both production and food safety challenges in viticulture systems (1, 9).

Future developments should focus on improving taxonomic resolution while maintaining analytical speed. Promising strategies include the integration of low-impact fluorescent probes, machine-learning-based classification of multidimensional cytometric datasets, and hybrid workflows combining rapid flow cytometric screening with targeted molecular confirmation. Additionally, miniaturization of flow cytometry platforms may facilitate their integration into field-adaptable monitoring systems, enhancing the potential for high-frequency aerobiological surveillance in precision viticulture. Importantly, the approach is inherently rapid and cost-efficient, enabling high-throughput analysis of large numbers of samples within a short time frame. These features make flow cytometry particularly suitable for integration into routine aerobiological monitoring and decision-support systems in viticulture.

From an applied perspective, the ability to rapidly detect airborne inoculum of key pathogens such as *B. cinerea* and *A. carbonarius* represents a significant step forward for disease forecasting. Early detection of spore presence in vineyard air can support more timely and targeted interventions, potentially reduce fungicide applications and limit mycotoxin contamination.

5. Conclusion

The present study provides a qualitative validation of flow cytometry for the detection of *B. cinerea* and *A. carbonarius* in spore trap samples collected from vineyard environments. By demonstrating the feasibility of rapid, low-cost, and reagent-minimal detection of key bunch rot pathogens, this work bridges the gap between high-throughput analytical technologies and practical aerobiological monitoring. Notably, the approach enables the processing of up to sixty samples per hour, significantly enhancing analytical throughput while maintaining operational simplicity and cost-efficiency. These findings support the potential integration of flow cytometry into disease forecasting systems, offering a promising alternative to conventional microscopy- and molecular-based approaches for real-time vineyard pathogen surveillance.

Declarations

Ethics approval and consent to participate:

• Not Applicable

Consent for publication:

• Not Applicable

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Author Contribution

E.C., A.A., E.A., S.P. conceptualized the work and developed the methodology; E.C., A.A., E.A. managed the data curation, formal analysis and investigation; the project was administrated by S.P., F.M., F.F.; S.P., F.N., F.M.

supervisioned the work; S.P., F.M. acquired the necessaries founds ; S.P., F.F., F.N., F.M. acquired the necessaries resources. The original draft was prepared by: E.C., A.A., E.A.; while all the authors reviewed the manuscript.

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Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

• Competing interests: The authors declare that they have no competing interests

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Figures

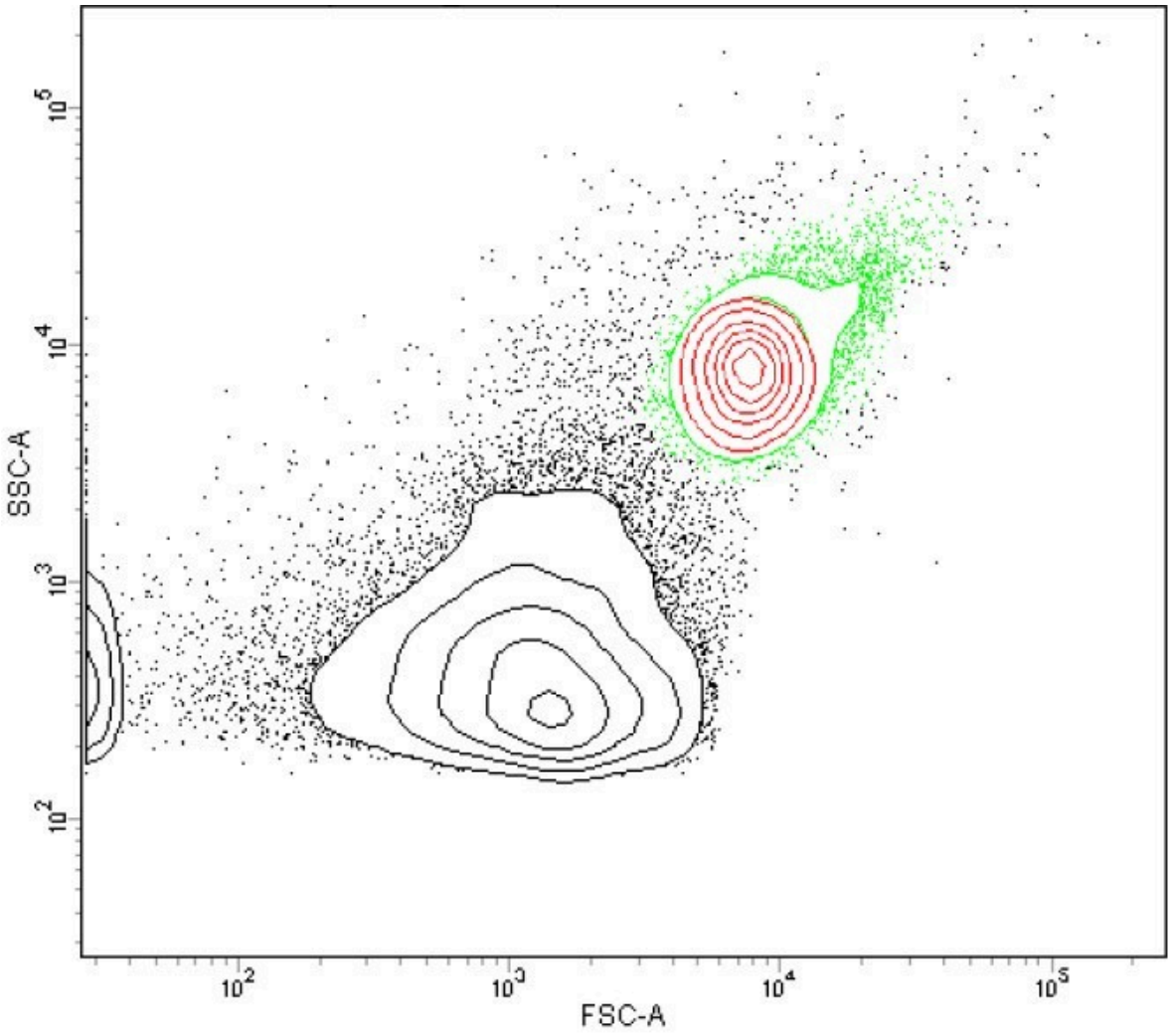


Figure 1

Aspergillus carbonarius *dot plot with all the detected events. In red, all events counted within the species-specific gate; in green, spores morphologically different from most of the population; in black, all the events not attributable to the fungal population*.

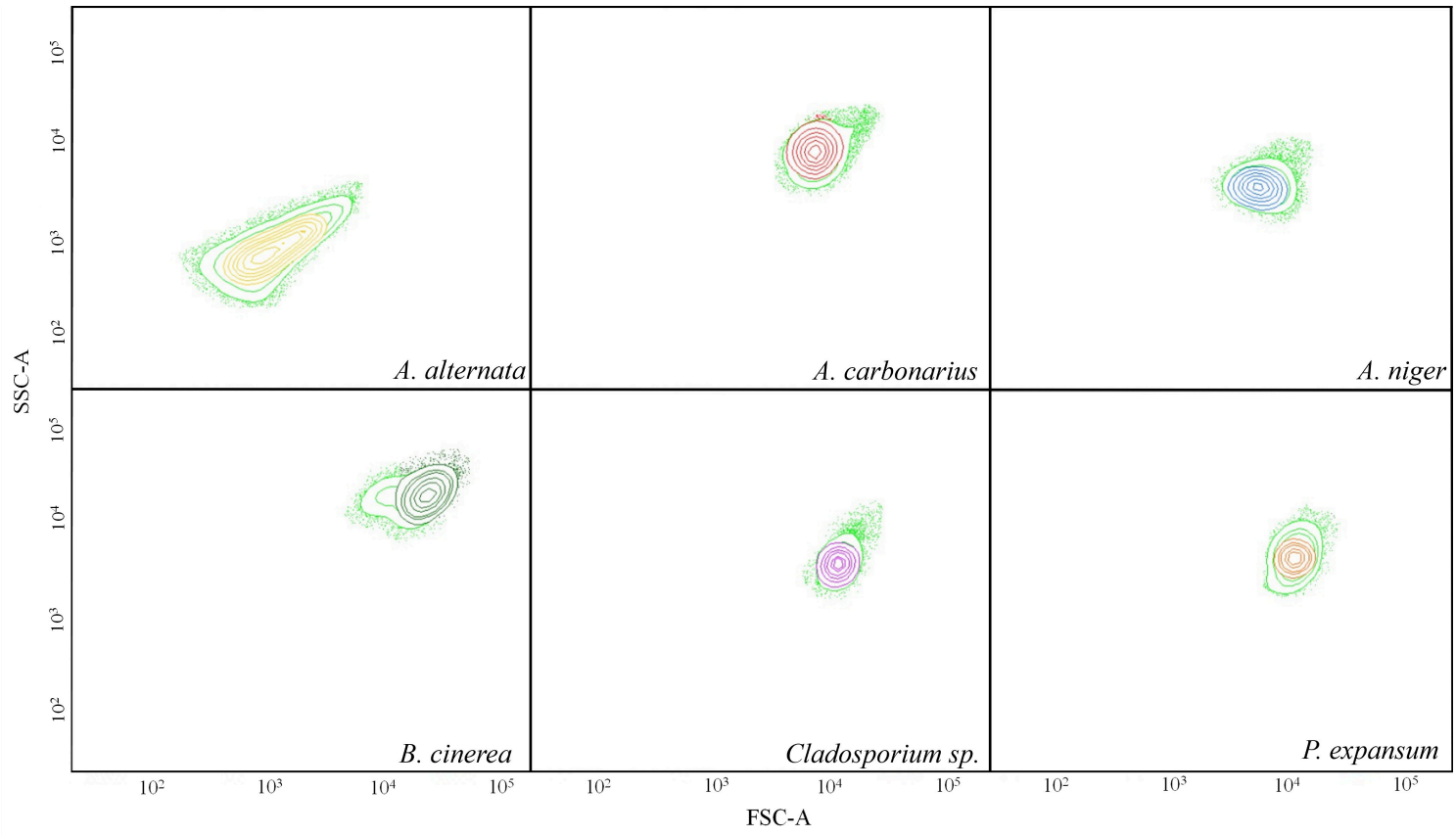


Figure 2

Morphological characterization of analyzed fungal specie conidia populations. Dot plots represent the morphological characteristics of fungal spore populations. Side scatter (SSC-A) is displayed on the y-axis and forward scatter (FSC-A) on the x-axis. Events associated with each population are highlighted in light green, while species-specific gates are overlaid in different colors: Alternaria alternata *(yellow),* Aspergillus carbonarius *(red),* Aspergillus niger *(blue),* Botrytis cinerea*(dark green),* Cladosporium sp. *(purple), and* Penicillium expansum*(orange).*

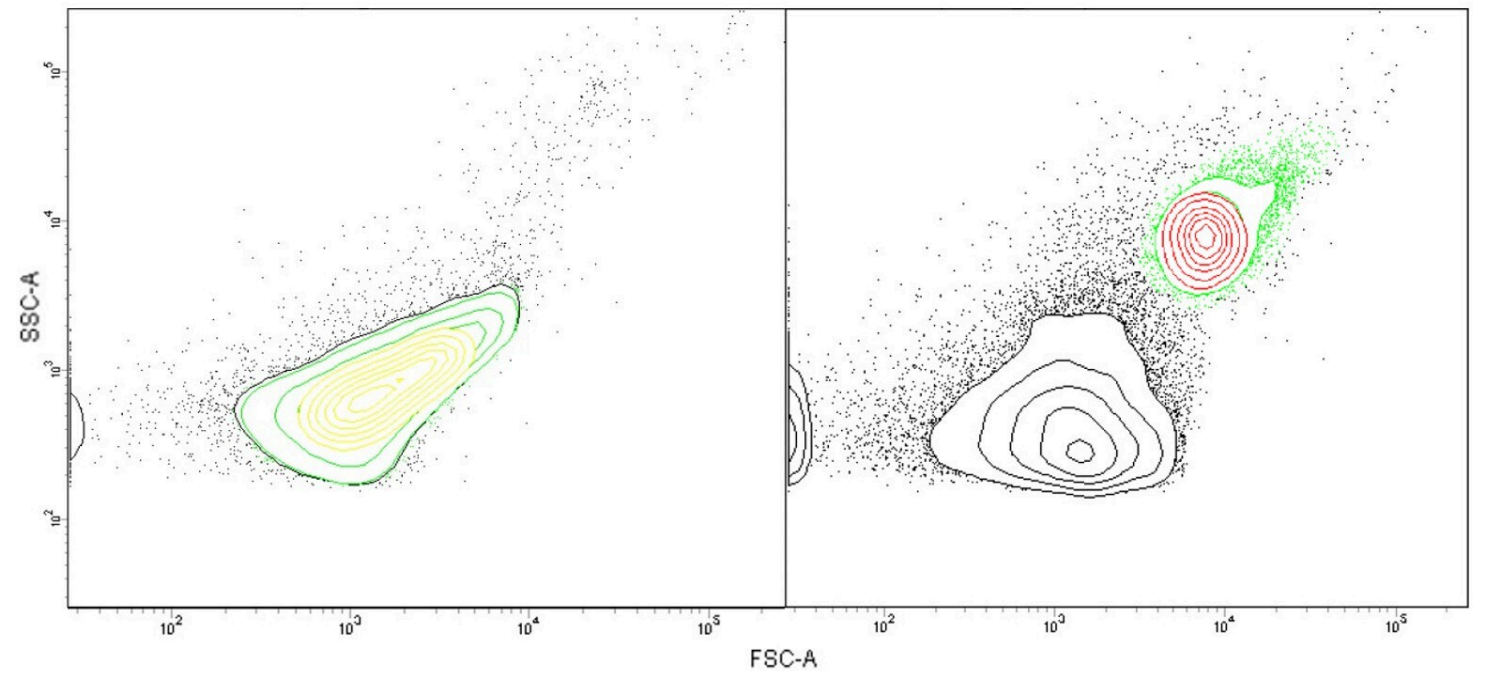
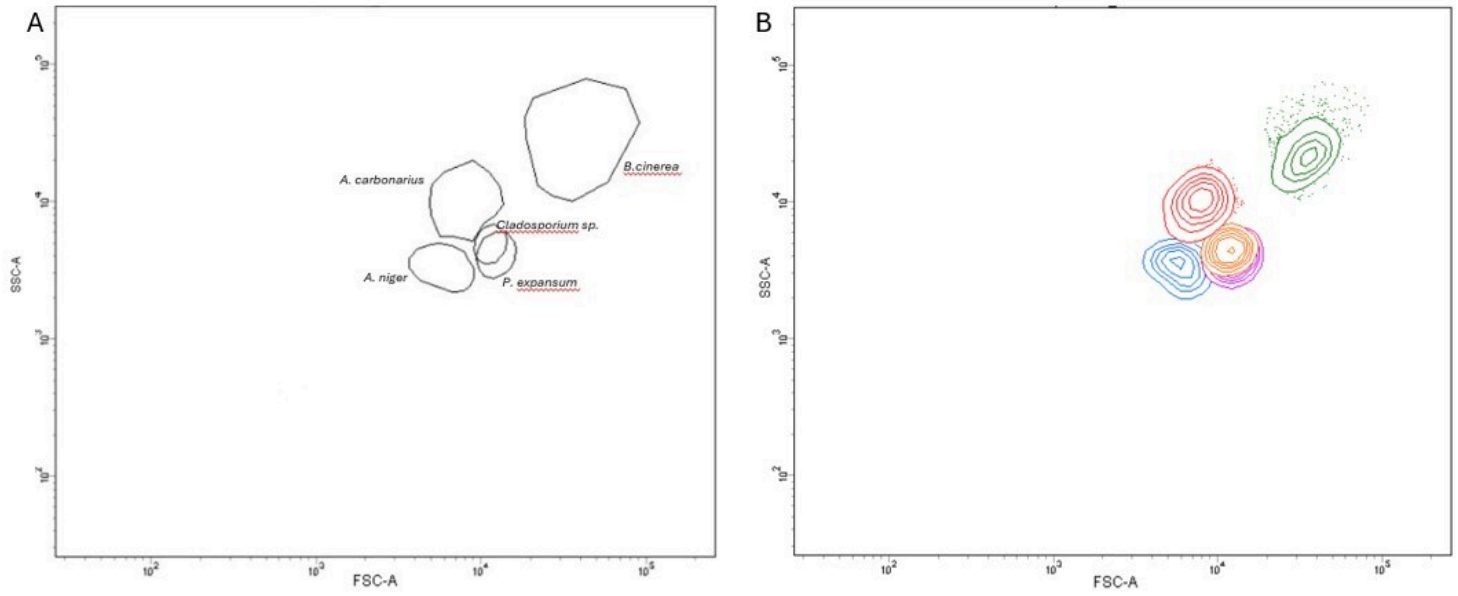


Figure 3

Impossibility to discriminate Alternaria alternata *conidia. Spatial overlap between the conidial population of* Alternaria alternata *(left) and debris present in the Aspergillus carbonarius suspension (right).*



C

	Percentage of recorded events in each specie-specific gate				
Mixed spores suspension	*Aspergillus carbonarius*	*Aspergillus niger*	*Botrytis cinerea*	*Cladosporium sp.*	*Penicillium expansum*
	8.5	6.2	7.8	5.7	8.6

Figure 4

Flow cytometry analysis of a mixed spore suspension. a) Gating strategy adopted in this study for the specific identification of spore populations of Aspergillus carbonarius*,* Aspergillus niger*,* Botrytis cinerea*,* Cladosporium sp.*, and* Penicillium expansum*. b) Results displayed in dot plot:* Aspergillus carbonarius *(red),* Aspergillus niger *(blue),* Botrytis cinerea*(dark green),* Cladosporium *sp. (purple), and* Penicillium expansum*(orange). c) Percentage of events falling within each gate after the analysis of the mixed conidial suspension.*