

Stimulation role of pollen grains in the initial development of *Botrytis cinerea*: The importance of host compatibility, cultivation status and pollen size

Ivett Kocsis  | Marietta Petrőczy  | Kolos Zoltán Takács | Gábor Markó 

Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

Correspondence

Gábor Markó, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, Ménése út 44, Budapest 1118, Hungary.
Email: marko.gabor3@gmail.com

Funding information

Thematic Excellence Programme 2020-National Challenges Subprogram, Grant/Award Number: TKP2020-NKA-16

Abstract

Plant pollens and fungal spores are massively produced in natural habitats and large-scaled agricultural areas, and the airborne transported biomass can be accumulated on relatively large crop surfaces. A pollen grain contains a specific chemical composition in quantity and quality, highly varied among plant species. So, the accumulated pollens could provide a species-specific nutrient supply for the initial fungal development in a pollen trap. Thus, this could increase the variation of the pathogen infection risk in certain plants due to selection mechanisms such as host–pathogen coevolution, crop breeding or pollination type. However, the role of the pollen-stimulated spore germination and the species-specific differences in the pollen-spore interferences is still less understood. Therefore, we performed a multispecies experimental comparison (including 20 plant species) to test the role of the taxon-specific attributes (i.e. host–pathogen compatibility, cultivation, pollination type and pollen size) in the dose and temporal response of the spore germination in *Botrytis cinerea*. We found a strong pollen-stimulating effect on spore germination; however, the triggering effect highly varied among plant species and differed between specific functional categories across temporal and concentration gradients. Specifically, the *B. cinerea* hosts, the non-cultivated plants and species with larger pollen size, increased the spore germination's stimulation effects, but not the pollination type. The systematic taxonomic and functional differences might reflect indirectly the diversity of the pollens' chemical profile (i.e. composition matrix or specific trigger molecules) which different selection mechanisms might shape. In the crop–pathogen interactions, the pollen-stimulation effect could play an essential role in early fungal development.

KEYWORDS

Botrytis cinerea, grass, grey mould, plant–pathogen interaction, pollen extract, pollen trap, pollen–spore interaction, spore germination, weed

1 | INTRODUCTION

The airborne components fundamentally determine a wide range of biological processes (e.g. pollination) and often can be the source of several adverse environmental, agricultural and human impacts (Randall, 1990). The vast majority of the airborne organic mass is plant pollen grains and fungal spores, which are often transported together (Magyar, 2008). However, a considerable amount of evidence has been collected regarding the pollen and spore airborne transportation and related issues; there is still a lack of information about the biological processes after the accumulation of the pollen and spore grains, especially their interspecific interactions.

The natural pollen trap is a specific surface of an inanimate object or a living organism where the accumulation of atmospheric plant pollens takes place (Zhang, 2018). However, the term of pollen trap is mainly referred to the deposit place of plant pollens, other airborne particles with similar sizes could also be deposited massively, such as fungal spores (Magyar, 2008). So, we used this term as an accumulation place for both pollen and spore hereafter. The plant surface can be a very effective pollen trap if the particular organ has a relatively large surface with depressions and complex structures, such as leaf stalks, flowers, fruits or bark (Fægri et al., 1989; Groenman-van Waateringe, 1998; Song et al., 2014), and can also preserve surface moisture and wetness (Sosa-Alvarez et al., 1995). A pollen trap containing a considerable amount of pollen and other organic materials with moisture could provide an ideal microenvironment, food source and chemical trigger molecules for spore germination and the initial fungal growth (Hennebert, 1973). A crop plant is often exposed to a massively produced pollen by the cultivated or other plants and spore grains due to the sporulation of the crop-specific plant pathogens. Furthermore, the pollen trap effect may be even more pronounced in a crop plant growing in large-scale agricultural areas than in a plant living in a natural habitat. Therefore, if a pollen trap accumulates pathogenic fungus spores and creates ideal environmental conditions to support the initial development of specific pathogens, a pollen trap could be a potential infection risk zone for the plant pathogen infection depending on the host-pathogen compatibility.

The stimulatory effect of pollen grains on conidia germination and initial colonization was barely documented previously, focusing on a few plant-pathogen pairwise comparisons. However, these early studies only revealed the presence or absence of the stimulation response in the particular pollen-spore interaction (Bachelder & Orton, 1962; Chou & Preece, 1968). These early studies showed clear evidence for the spore stimulation by exposition of the pollen's aqueous extracts, which were exposed to different concentration levels, but the comparison of these results was challenging due to the heterogeneity of the applied methods among studies. The high variation of the described concentration thresholds suggested a powerful species-specific stimulation effect in the plant-pathogen interactions. Similarly, other studies reported also high variance in the pollen chemical composition within- and between species or pollination types (Kostić et al., 2017; Nicolson et al., 2018; Nicolson &

Human, 2013). Thus, the chemical composition could be a potential source of the high heterogeneity of the pollen-spore interactions. At the same time, these patterns could also be driven by the consequences of the specific host-pathogen coevolution and breeding processes which could be the primary selection forces shaping the pollen variation in crop plants' morphology and physiology (Lyu et al., 2021; Occhipinti, 2013; Piskorski et al., 2011). Thus, these factors may also play an important role in the pollen stimulatory effect generating differences between host and non-host or crop and non-crop plants. These species-specific and functional attributes may explain the heterogeneous pollen-spore interactions, which urges the need to reveal these unknown mechanisms using a multispecies study approach.

Regarding the potential role of the pollen-spore interaction in the initial development of specific pathogens, studying the pathological risk of plant pollen exposition would not be essential only for a theoretical purpose, but it could be helpful for practitioners in crop production. These effects could be more emphasized in those plant pathogens of which spores transferred and accumulated together with pollens, and so the airborne pollens could constantly stimulate the conidia germination in cultivation, such as in *Botrytis cinerea* Pers (Chou & Preece, 1968; Ogawa & English, 1960). The causal agent of grey mould is *B. cinerea* Pers, a facultative parasite fungus, being a significant pathogen with a wide range of host plants, causing critical yield losses worldwide year by year in several cultivations such as strawberry (*Fragaria × ananassa*), raspberry (*Rubus idaeus*) and grape (*Vitis vinifera*). Airborne conidia of *B. cinerea* are produced intensively and, as a primary source of inoculum, can infect plant organs, such as flowers and fruits (Romanazzi & Feliziani, 2014). Moreover, these conidia are transported through advection and can be easily trapped in natural pollen traps on the surface of crop plants. These species-specific features and the positive conidia germination response to the pollen extract make *B. cinerea* an ideal model organism to test the general patterns of pollen-spore interaction.

The pollen grains have a species- or function-specific chemical composition profile (Hesse, 1981; Nicolson & Human, 2013), so their aqueous extracts could generate a high variance among plant taxa along with the pollen concentration and the temporal gradients in the conidia germination (Chou & Preece, 1968). Therefore, in this multispecies comparison, we paid particular attention to the species and function-specific pollen attributes which could interfere with the pollen-spore interactions generating differences in the concentration- and temporal-dependent stimulatory effect on conidial germination in *B. cinerea*. Specifically, we tested the possible role of the species-specific functional characteristics such as host-pathogen compatibility (non-host or host), cultivation (non-crop or crop), pollination type (anemophilous or entomophilous) and pollen size. Regarding the tested factors, we formulated the following predictions. First, due to the long-term coevolutionary background between the host plants and their pathogens by chemical composition-based cue recognition mechanisms (Rezzonico et al., 2017), we hypothesized that pollen from the host plant of *B. cinerea* has a more substantial effect on conidia germination than an incompatible plant. Second,

we predicted that a crop plant has a more significant stimulating effect on *B. cinerea* conidia than a non-crop, a weed or free-living species because the selective breeding processes could affect the pollen morphological attributes and chemical compositions. Third, we hypothesized functional and chemical differences between anemophilous and entomophilous plants, which may explain the variance in stimulatory effect. Fourth, if the pollen variances in the chemical composition determine the temporal dynamics of conidia germination, we assumed that pollen with greater biomass contains more stimulants and/or represents a higher nutrient value for the initial conidia development, which may influence conidia germination more effectively. Therefore, in the present study, we provided a detailed overview of the pollen-spore interaction to deepen our understanding of the pollen stimulatory effect in *B. cinerea*.

2 | MATERIALS AND METHODS

2.1 | Pollen collection, isolation, identification and maintenance of the pathogen

Testing biologically relevant pollen-spore interactions, we targeted to test specific pollinating plants potentially living in agricultural or rural habitats and overlapping their flowering period with the susceptible phases of the potential host infection by *B. cinerea*. The susceptible phenological stage to *B. cinerea* infection was defined based on the vegetation of strawberry, raspberry and grape hosts. At the same time, the overlapping flowering periods were explicitly selected based on a long-term descriptive national survey (Udvardy et al., 2019). Additionally, we selected plants with high pollen production categorizing them into different functional groups. Specifically, the tested species included host plants of the pathogen and non-host plants, anemophilous and entomophilous plants, and non-crop and crop species (e.g. cover plants and weeds).

To extract fresh pollen for laboratory analyses, we collected flowering plant parts in full blooming from the focal species based on the species-specific blooming periods (between May and September) in two consecutive years (2018, 2019) in agricultural habitats (for sampling details, see Table S1). The plant samples were labelled individually and transported into the laboratory for further preparations. Only the freshly opened flowers were selected to extract the pollen grains manually in the laboratory, attempting to collect 1–1.5 cm³ pollen grains in each species. Then, the pollen grains were stored in a paper envelope (7°C) for further use. To approximate the pollen size, we estimated the pollen grain volume by measuring the polar and equatorial diameters of pollen grains ($N = 10$ in each species with the accuracy of .1 µm) and calculated the spherical volume according to Dimou et al. (2020). The measurement was carried out under a light microscope (Nikon Eclipse 50i) at 400× magnification using QCAPTURE Software (version 6.0). Next, we created a derived variable called 'volume corrected concentration' after multiplying the values regarding species-specific pollen, approximate pollen volumes and the pollen concentration (zero, medium and high, see later). This

variable can express the pollen exposition in the aqueous extracts more accurately than the concentration alone.

In the laboratory, we established a *B. cinerea* strain isolated originally from an infected bunch of grapes (cv. *Furmint*) collected nearby Szekszárd (Hungary). The applied *B. cinerea* strain (BC2108; Institute Type Collection, Hungarian University of Agriculture and Life Sciences, Institute of Plant Protection, Budapest, Hungary) was previously analysed, including morphological features, cultural characteristics, pathogenicity tests and molecular tools also confirmed the identification. Conidia were cultured on PDA plates (Biolab Inc.) with five replications at 24°C in the dark. On the 14th day of the incubation period, colony and conidial morphology were visually observed and checked. Morphological identification was performed according to Coley-Smith et al. (1980). The *B. cinerea* cultures were maintained on malt extract agar (Green et al., 2006). Before each pollen-spore test, the conidia were harvested manually from the sporulating part of the colony in a sterile environment.

The pathogenicity test was conducted on mature grape berries (cv. *Furmint*) with conidia in three parallel replications. First, the surface-sterilized berries with pedicels were placed in sterilized glass containers on moist filter paper. After that, the conidial suspension (4×10^4 conidia/ml) was added dropwise to the surface of the fruits. The inoculated berries were incubated with 95%–100% relative humidity at room temperature ($21 \pm 2^\circ\text{C}$). Finally, *B. cinerea* was re-isolated from the necrotic tissues to confirm Koch's postulates (data not shown).

2.2 | Measuring pollen-stimulating effect

Firstly, three different pollen suspensions were prepared with different concentrations, including zero, medium and high (0, 5×10^4 and 1×10^5 pollen grains per ml, respectively). The proper concentration was validated by using the Bürker chamber. After the validation process, the suspension was allowed to stand for 18 h for the dissolution of the pollen constituents at room temperature ($21 \pm 2^\circ\text{C}$) (Békési, 1977). Only the supernatant fraction (aqueous pollen extracts without containing pollen grains) was added for stimulation. Secondly, the sporulating *Botrytis* culture was washed with sterile water to harvest the produced conidia. After validation, we applied a standard concentration of conidia suspension in all tests (5×10^5 conidia per ml).

The pollen-spore interaction test started when the aqueous pollen extract was added and gently mixed with the conidia suspension in a ratio of 1:5 (.08 and .4 ml, respectively) onto the microscopic slides with reaction wells (Omano, diameter depression 15 mm). The microscopic slides were then placed into sealed sterile Petri dishes for incubation to maintain the humid and sterile environment. Three microscopic slides were prepared as repetitions in each pollen concentration, and we examined them separately in each plant species. Conidia germination was measured at 4, 6 and 8 h after the beginning of the treatment by visual observation using a light microscope (Nikon Eclipse 50i) at 400× magnification. We examined through

five microscopic views in each sampling event, counting 10 random conidia. A conidium was considered to be germinated if the germ tube length reached or exceeded the largest diameter of the conidia. Finally, we expressed our observations as a percentage of the germinated conidia in each sampling unit and used them as a response variable in the statistical analyses.

2.3 | Statistical analysis

Merely for validation of the applied measuring procedure, we quantified the repeatability as the ratio of the within-group and the total residual variances calculated by Markov Chain Monte Carlo sampler for multivariate Generalized Linear Mixed Models based on repeated measurements of the identical sample units (R package: 'MCMCglmm', Hadfield, 2010). The mean and the quantiles (2.5% and 97.5%) of the MCMC outputs represented the repeatability index and the confidence intervals (CI_{95}), respectively, based on 1000 permutations. As a result, we derived a high repeatability index (.701; CI_{95} : .681/.721), which means that data of independent measurements carried out under the same conditions were in close agreement. This result indicated meaningfully that our test series followed a valid methodology. First, we tested the basic concentration- and temporal-dependent patterns on the germination ratio by a Generalized Mixed Effect Model with a negative binomial error structure (GLMM-nb) approaching the distribution of the response variable. In this 'basic' model, we entered the percentage of the germinated conidia as the response variable, and the predictor variables were the concentration of the pollen extract ('concentration'), the measuring time ('time') presented as covariates and their interaction (concentration $\times$ time). At the same time, the random factors represented the slide identification number due to the repeated measuring of the identical slides, and a hierarchical nested random structure (family nested in the order) provided a statistical control to the botanical origin.

Second, we tested the species- and functional-specific related predictors such as 'Species', 'Host compatibility', 'Cultivation' and 'Pollination' (as factors). The basic GLMM-nb model structure was supplemented with a single predictor one by one in each model and running them separately. Integrating all the tested variables into a single model could not be performed because each predictor carried a species-dependent attribute generating zero variances. We applied a likelihood ratio test (LRT) to test each predictor's effect in these extended models. After that, models with significant predictors were further analysed by testing the specific interactions such as concentration $\times$ predictor and time $\times$ predictor.

Final, we tested the effect of pollen volume on the pollen-spore interactions. As a first step, we tested the presence of an a priori systematic variance of the pollen volume between the functional groups by Welch's *t*-test. If the functional groups were the same, we could consider the pollen volume as a separate, species-specific attribute. Next, we tested the effect of the pollen volume-corrected concentration ('Concentration-volcorr') using a separate GLMM-nb

model. In this model, we entered the germinated conidia ratio (per cent) as the response variable, and the 'Time' and the 'Concentration-volcorr' presented as covariates, and their interactions, while the random factors were the same as in the basic model.

The numeric predictors were centred and scaled in all statistical models before running the analyses. The model diagnostics were always checked in all final models. The post hoc tests were calculated by pairwise Student's *t*-test comparisons between group levels with 'Holm' adjustments of the *p*-values due to the planned multiple testing. The statistical analyses were carried out in the R statistical environment (version 3.6.2, 12-12-2019, R Development Core Team, 2019) using the 'lme4' packages (Bates et al., 2015).

3 | RESULTS

To explain the variation of the pollen aqueous extracts' stimulation effect on the spore germination in *B. cinerea*, we tested the species- and several function-specific attributes such as host compatibility, cultivation, pollination type and pollen volume.

3.1 | Overall impact

First, we found robust and general concentration and temporal-dependent patterns in the germination ratio (Figure 1). The spore germination increased with the measuring time along with the concentration gradient of the aqueous pollen extract. The significant interaction between time and concentration suggested non-zero temporal dynamics in the germination ratio among the concentration treatments.

3.2 | Functional attributes

Second, we tested the specific functional attributes by expanding the basic model using a focal predictor. We found that the species, host compatibility and cultivation highly affected the germination ratio, while the pollination type was unaffected. In all expanded models, the basic relationships regarding concentration and temporal patterns were consistently the same as we revealed previously in the basic model (Table 1).

Focusing on the botanical differences (Figures 1 and 2), we detected high species-specific variations, including weak (e.g. *Medicago sativa*, *Elymus repens*), medium (e.g. *Fragaria $\times$ ananasa*, *Sambucus nigra*) and strong (e.g. *Vitis vinifera*, *Cupressocyparis leylandii*) stimulation effects. Furthermore, this general level of the spore germination showed considerably different temporal dynamics in germination along the concentration gradients (for more details, see Table S2).

Interestingly, we could detect spore stimulation effects in most non-host and non-crop species, and only a few pollen extracts (with non-zero concentrations) caused undetectable responses. Specifically, in the host species, the medium and high

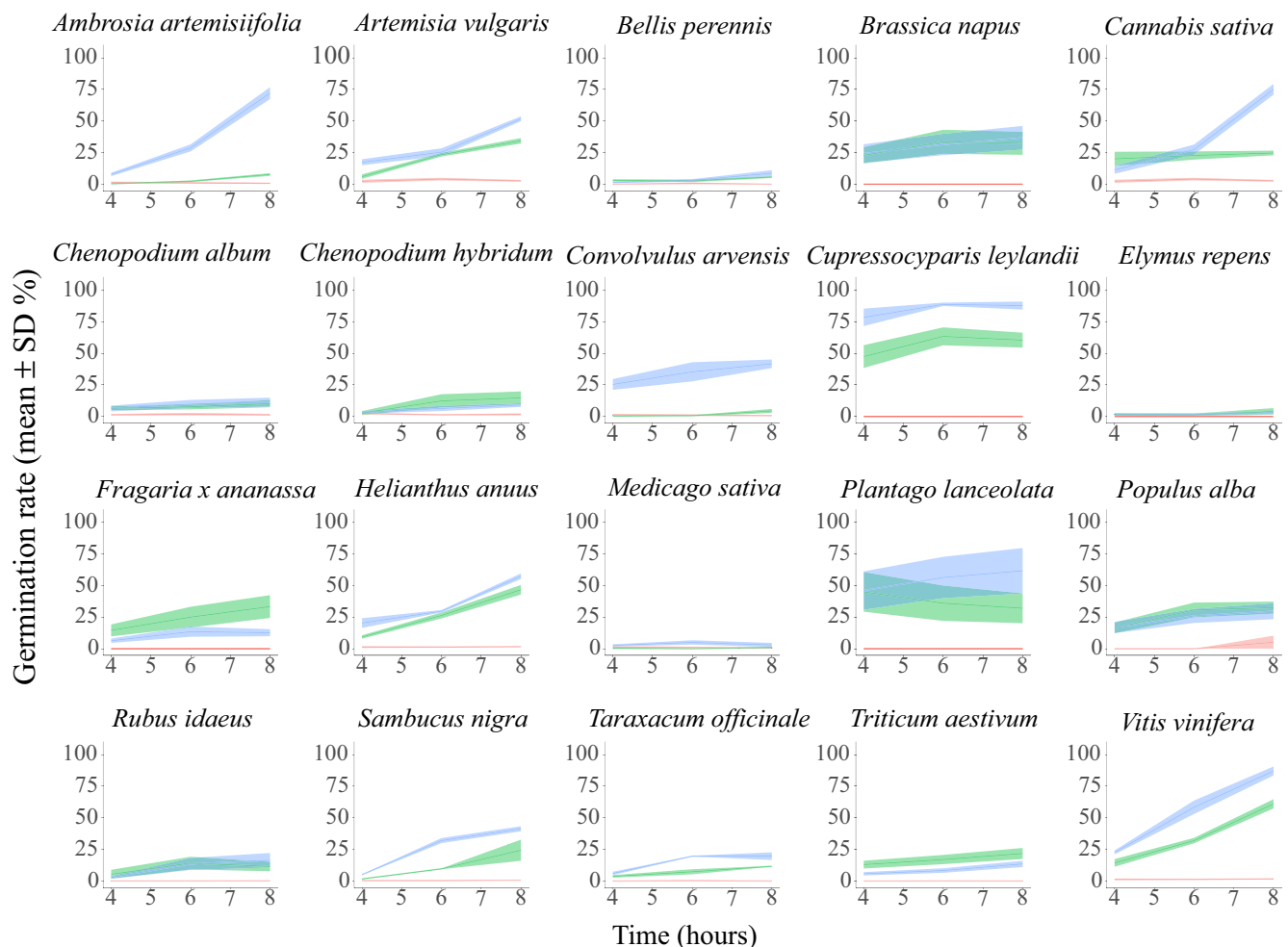


FIGURE 1 Plot summarizes the species-specific stimulation effect of the pollen aqueous extracts on the conidia germination rate within the measuring period along with three different concentration levels. The lines with different colours represent the different concentration treatments of the aqueous pollen extracts (red: zero, green: medium, blue: high concentration). The shaded zones around a line indicated the standard deviation of the corresponding mean value.

pollen concentration extracts stimulated a similarly high and consistent temporal increase in the germination ratio (Figure 3, Table 1, Tables S3 and S4). In contrast, the non-host pollen extracts showed a concentration-dependent response at the medium and high concentration treatments 8 h after the tests started. Similarly, a stimulation difference emerged in the crop and non-crop comparisons, with a difference appearing after 6 h between the medium and high treatments in the crop plants, while in the non-crop species, this effect was consistently the same in the non-zero concentration groups throughout the study.

3.3 | Pollen volume

Third, we tested the effect of pollen volume on the pollen-spore interactions. Nevertheless, before testing a quantity feature, we tested whether there was a priori systematic variance in the pollen volume between the functional groups, which could result from differential selection environments. Then, we confirmed that all

functional groups were homogenous in pollen volume (Host compatibility: $t = -1.217$, $df = 16.14$, $p\text{-value} = .24$; cultivation: $t = .399$, $df = 15.06$, $p\text{-value} = .69$; and pollination: $t = .264$, $df = 17.51$, $p\text{-value} = .79$). So, we could test this species-specific feature as a separate attribute. Similarly to previous models, we found a temporally consistent stimulation effect and detected a robust increase with the exposition of the volume-corrected concentrations (Figure 4, Tables S1 and S5).

4 | DISCUSSION

This study investigated the pollen's possible role, which could effectively interfere with the pollen-spore interactions. We compared 20 plant species with functionally diverse attributes and estimated the stimulation effect of their aqueous pollen extracts on spore germination in *B. cinerea* as a polyphagous and widely distributed fungus. In general, we found robust concentration- and temporal-dependent stimulatory effects, which highly varied among species.

Model name	Predictor variable	Chi ²	df	p-Value
Basic	Time	135.677	1	<.001***
	Concentration	296.148	1	<.001***
	Time × concentration	9.128	1	.002**
Species	Time	147.287	1	<.001***
	Concentration	332.129	1	<.001***
	Species	148.045	19	<.001***
	Time × concentration	11.248	1	<.001***
	Concentration × species	48.760	19	<.001***
	Time × species	47.529	19	<.001***
Compatibility	Time	137.098	1	<.001***
	Concentration	297.792	1	<.001***
	Host compatibility type	4.413	1	.036*
	Time × concentration	9.968	1	.002**
	Concentration × host compatibility type	2.249	1	.133
	Time × host compatibility type	4.694	1	.03*
Pollination	Time	135.557	1	<.001***
	Concentration	297.209	1	<.001***
	Pollination type	1.413	1	.234
	Time × concentration	9.088	1	.003**
Cultivation	Time	136.064	1	<.001***
	Concentration	306.461	1	<.001***
	Cultivation type	8.699	1	.003**
	Time × concentration	9.505	1	.002**
	Concentration × cultivation type	5.347	1	.021*
	Time × cultivation type	.601	1	.438
Pollen volume	Time	140.879	1	<.001***
	Volume-corrected concentration	69.120	1	<.001***
	Time × volume-corrected concentration	.006	1	.941

TABLE 1 Results from generalized mixed effect model with a negative binomial error structure (GLMM-nb), in which the conidia germination was the response variable, testing the association with specific predictors and their interactions.

Note: The chi-square values, the degree of freedom (df) and the significance levels (p-value) were calculated from the corresponding likelihood ratio test that compared the model fit of the full model and the reduced model after excluding the given predictor. The stars indicated the level of significance of the given predictor (* $p < .05$, ** $p < .01$, *** $p < .001$).

Furthermore, the dose–response patterns reflected a considerable high species-specific pollen difference in the response's magnitude and speed (Figures 1 and 2, Table S2). These results supported the previous assumptions that pollens might bear a unique chemical composition which could generate species-specific differences as germination stimulants of fungi spore.

Regarding small water-soluble molecules, the results provided indirect evidence for the validity of the basic assumptions that the pollen grains' chemical profile and their taxonomic variance in quality and quantity could play an essential role in the interspecific interactions. Generally, pollen and other pollen-based food products were often considered high in nutrient and vitamin values due to the specific carbohydrates and protein compositions (Nicolson &

Human, 2013). In close agreement with our results, similar species-specific chemical composition patterns (e.g. carbohydrates, proteins, lipids and trace elements) were revealed in different plant pollens and pollen-kitts (Dobson, 1988; Piskorski et al., 2011; Stanley & Linskens, 1974). Recently, only a handful of studies attempted to describe a detailed chemical composition of the hand-collected pollens, mainly for the descriptive purpose of their nutrient values (Kostić et al., 2017; Nicolson et al., 2018; Nicolson & Human, 2013).

The natural selection and crop breeding processes can generate a driving force for the fitness-related traits shaping systematic functional differences among plants, such as fertilization and the pathogen infection (Occhipinti, 2013). So, these selection forces could also affect the pollen's chemical and morphological attributes.

FIGURE 2 Violin plot illustrates the general species-specific stimulation effects of the pollen aqueous extracts on the spore germination rate of *Botrytis cinerea*. In each species, the vertical black line represents the interquartile data range, while the points indicate the mean and median germination ratio by a red circle and a green square, respectively. The different letters show significant differences at $p < .05$ level according to the post hoc tests. The icons represented the species-specific attributes (host compatibility: host, non-host; cultivation: crop, non-crop; pollination type: anemophilous, entomophilous).

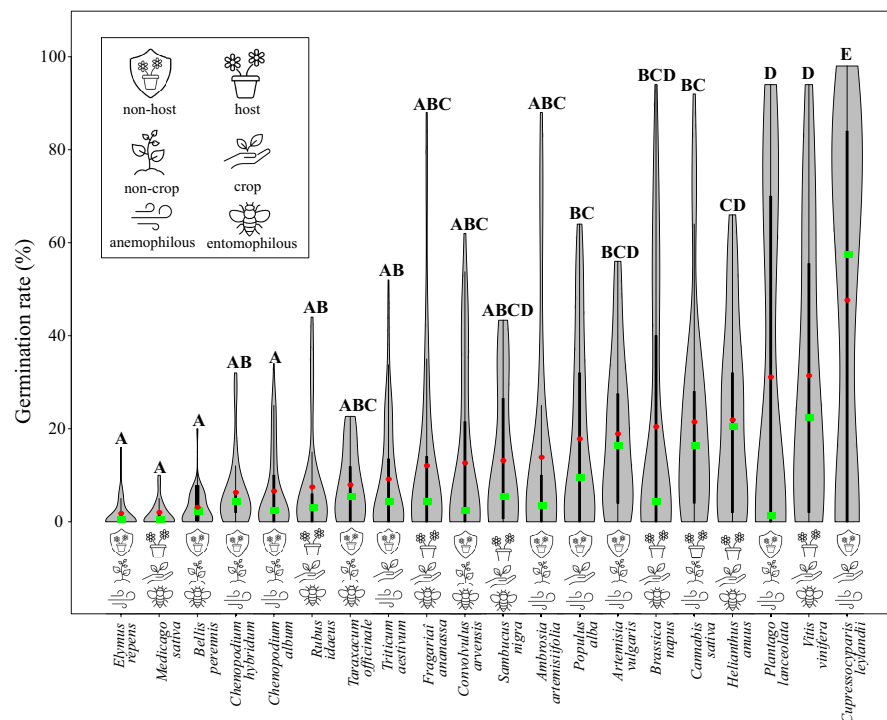
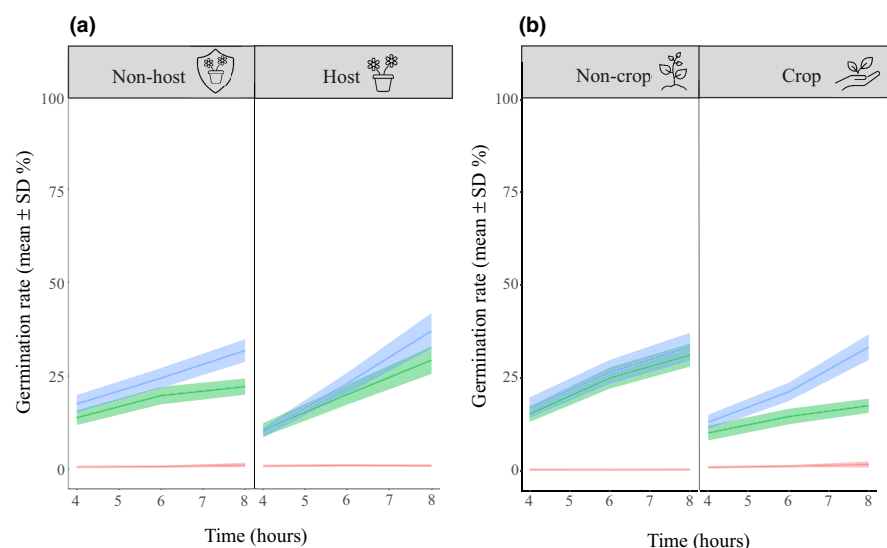


FIGURE 3 Plot shows the conidia germination rate's temporal- and concentration-dependent patterns regarding the host compatibility (a) and cultivation types (b). The lines with different colours represent the different concentration treatments of the aqueous pollen extracts (red: zero, green: medium, blue: high concentration). The shaded zones around a line indicate the standard deviation of the corresponding mean value.



Therefore, we predicted that if the pollen morphological structure and chemical composition were shaped by the same long-term selection process, such as host-pathogen coevolution, plant breeding or pollination, we could detect similar systematic variation in the dose-response spore stimulation among taxa within the same functional group. Supporting the previous expectations, we detected systematic differences in the functional attributes, such as host-pathogen compatibility and the type of cultivation (Figure 3, Table 1, Tables S3 and S4), but no differences between the pollination types. Thus, the non-random spore responses indirectly reflected the species- and function-specific differences via the pollen extracts' chemical composition profile. If so, the chemical profile within the same functional group would be more similar expectedly than between groups which prediction would be worth studying in the future.

Focusing on the relevance of the host-pathogen compatibility, the spore responses reflected taxonomic differences, which might be associated with the host-pathogen coevolution due to the differential qualitative and quantitative pollen properties. Specifically, however, both compatibility groups elicited stimulation effects in all non-zero concentrations, and the efficiency of the non-host pollen extracts was reduced than the extracts of the host's pollens (Figure 3, Table 1, Tables S3 and S4). An essential selection factor in a host-pathogen interaction is the hosts' chemical recognition of a pathogen and how effective and sensitive this system is, in which a plant pathogen could exert considerably high selection pressure on their host plants (Occhipinti, 2013). If such a process is under selection, we expected that a pathogen fungus could gain adaptive benefits by increasing the efficiency of the host recognition, generating

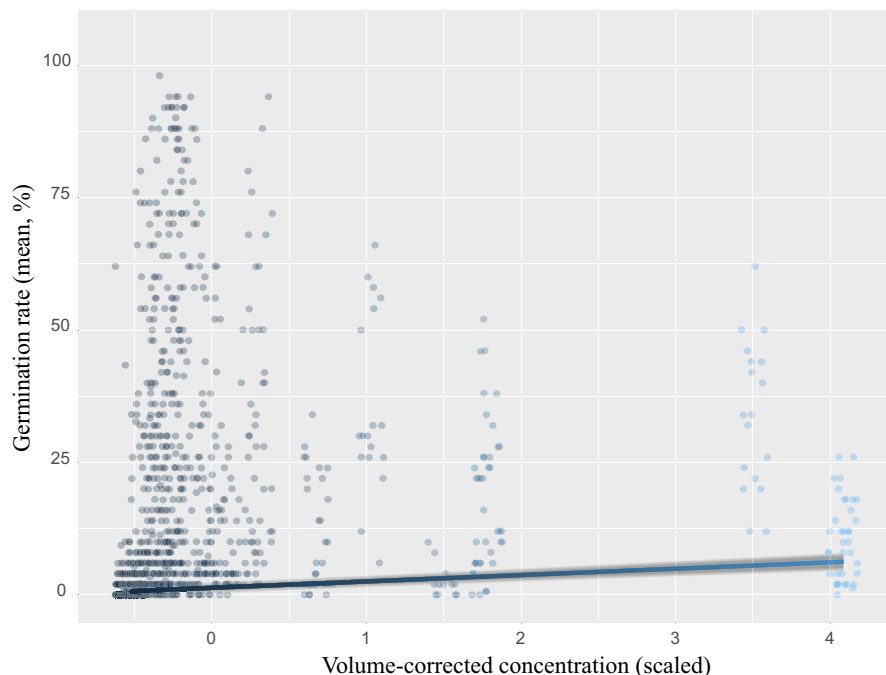


FIGURE 4 Plot shows the relationship between the volume-corrected concentration (scaled data shown) on the conidia germination rate (mean, %). The line represents the regression slope with the confidence intervals (shaded zone). The data were slightly jittered to improve the visualization.

a similar response at a reduced concentration of the chemical stimulants, as illustrated in the results of this study similarly. On the contrary, plants also developed strategies to cope with their pathogens, such as evolving new chemical compounds (e.g. organic acids) for masking host recognition of the pathogens (Lyu et al., 2021).

Crop breeding could also trigger directional phenotypic changes, evolving beneficial changes for plant management, while less direct selectional forces shape the non-crop plants occurring in natural or semi-natural environments (Wink, 1988). Despite the expected chemical diversity in pollens, this study revealed systematic variations in the stimulation effect between crop and non-crop groups. Like the effects of host-pathogen compatibility, crop and non-crop groups triggered the spore stimulation at medium and high concentrations (Figure 3, Table 1, Table S4). However, the effect of non-crop plants generates a more intense response at medium concentration than the effects of the crop plants at a similar treatment. These relationships supported accepting the predictions that breeding processes could also generate differential phenotypic variations providing positive benefits for the crop plants and reducing the harmful interference of the host-pathogen interactions.

The pollination type can fundamentally determine the pollen's morphological structure and chemical composition. For example, anemophilous plants always produce powdery pollen, while the pollen grains produced by entomophilous plants are mostly sticky, facilitating attachment to the pollinator (Hesse, 1981). Therefore, the effect of the pollination type was a biologically relevant functional attribute worth testing. However, besides the expectedly high variations between pollination types, the result showed that the pollination could not affect the conidia germinations. This pattern can be interpreted by the partially overlapped selection forces shaping the host-pathogen interactions than the processes regarding pollination differently. Therefore, this result suggested that the morphological

adaptations to a specific pollination form could not be enough to increase the spore stimulation effects. So, the potential role in spore stimulation could be less relevant.

Finally, we tested the hypothesis that the stimulation of the conidia germination increases with the pollen size, assuming that a larger pollen grain contains more nutrients and other stimulants than a smaller one (Stanley & Linskens, 1974). According to the expectations, the results showed that the effects were more pronounced in taxa producing larger pollen grains than plants with smaller ones (Figure 4, Table 1, Tables S1 and S5). Therefore, the pollen volume could be considered as a relevant species-specific attribute that varied independently on the variations related to host-pathogen compatibility, the cultivation or the pollination types. Moreover, similar to previous results (Stanley & Linskens, 1974), this relationship also suggested that the spore stimulation effect of pollen could be based on qualitative and quantitative indicators due to the consistent and robust dose-dependent relationship and the high species-specific variances, respectively.

This study provided highly varied pollen-spore interactions between certain relevant plant species and a fungus, *B. cinerea*. The results agreed with previous studies, which only demonstrated the presence or absence of the pollen-spore interactions between certain fungi and plant species (Chou & Preece, 1968; Fokkema, 1971; Ogawa & English, 1960), but our detailed multispecies study showed that the spore stimulation by plant pollens could be regulated by a more complex mechanism than previously expected. Therefore, further studies should pay particular attention to these small, water-soluble precursor molecules that could be responsible for regulating specific biochemical mechanisms. In addition, these pollen components might be structurally and functionally analogous to specific precursors in the spore, which could cause interference in similar processes during plant fertilization and spore germination.

In a comprehensive ecological approach, the plant organs (i.e. leaves, pollen grains) could provide unique habitats (e.g., not only as chemical environment or nutrient sources) for specific microbiome assemblages composed of members from the fungal and bacterial kingdoms (Fokkema, 1971; Junker & Keller, 2015; Manirajan et al., 2018). Thus, the species-specific host–pathogen compatibility could also be driven by the diversity of the epiphytic and endophytic microorganism assemblages, which could shape the effectivity of the natural plant-defence systems against pathogens via their intra- and interspecific competitions (Manirajan et al., 2018; Vandenkoornhuyse et al., 2015). However, so far, the role of the microbiome structures and associations is still less known in the specific host–pathogen interactions, which knowledge would be beneficial in agricultural practice.

In summary, the results of the present study suggested a non-random taxonomic variation as a consequence of the differential selectional forces, which might be manifested via the quality and quantity of the pollen chemical profile, and these effects might be the fundamental source of the heterogeneity in the spore–pollen interactions. Thus, a pollen trap could be an ideal environment for inter-specific interactions via pollen and spore. However, the role of these specific molecules and other influential factors are still unknown, which could be essential in these biological mechanisms. Furthermore, the revealed mechanism in the plant–pathogen interactions may have many practical implications for crop production and protection management. For example, the pollen–spore interactions could generate additional infection risks in crop plants as natural pollen traps by the pollen-sourced stimulating factors. Therefore, future studies should pay more attention to revealing the functional comparisons regarding the form of cultivation, host–pathogen compatibility or pollen size, especially in the initial development of plant pathogenic fungi.

AUTHOR CONTRIBUTIONS

IK: Methodology, Formal analysis and investigation, Writing the first draft, Review and editing; MP: Conceptualization, Methodology, Review and editing; KZT: Formal analysis and investigation; GM: Methodology, Formal analysis and investigation, Writing the first draft, Review and editing, Supervision.

ACKNOWLEDGEMENTS

This research was supported by the Ministry for Innovation and Technology within the framework of the Thematic Excellence Programme 2020-National Challenges Subprogram (TKP2020-NKA-16) for research on adaptation to the changing environment in the viticultural sector.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

ORCID

Ivett Kocsis  <https://orcid.org/0000-0002-1653-2154>

Marietta Petrőczy  <https://orcid.org/0000-0002-6139-8281>

Gábor Markó  <https://orcid.org/0000-0003-1351-4070>

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How to cite this article: Kocsis, I., Petrőczy, M., Takács, K. Z., & Markó, G. (2022). Stimulation role of pollen grains in the initial development of *Botrytis cinerea*: The importance of host compatibility, cultivation status and pollen size. *Journal of Phytopathology*, 00, 1–10. <https://doi.org/10.1111/jph.13149>