



# Cropping system support in downy mildew control in basil in organic farming: a two-year open field experiment

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## Abstract

Basil Downy Mildew (BDM), caused by the oomycete *Peronospora belbahrii*, is a major issue for sweet basil (*Ocimum basilicum* L.) production worldwide. Currently, the disease is mainly controlled by chemical fungicides, but the development of populations of the pathogen which are resistant to the most widely used compounds is leading to the research of alternative crop protection strategies. Therefore, in this paper, some cropping variables were tested in a field trial conducted in two consecutive years (2021 and 2022) in Northern Italy in organic farming conditions, with the overall objective to optimize basil productivity and quality and limit BDM occurrence. These include two basil varieties, two sowing densities (dense, 30 kg/ha, and sparse, 15 kg/ha), and two irrigation systems (drip and sprinkler). A higher incidence and severity of BDM in 2022 compared to 2021 was observed, mainly due to the different climatic conditions that occurred in the two years. Year 2022 was characterized by high temperatures and repeated drought phenomena that led to basil stress and BDM severe outbreak. Moreover, variety 1 (considered resistant to *P. belbahrii*) was confirmed to be completely resistant in 2021 but it was found to be susceptible the following year, with disease incidence and severity comparable to variety 2 (medium susceptible). No differences were detected in terms of BDM occurrence and crop yield between the two sowing densities (mean of 58.4% and 26.6% of BDM incidence and severity, respectively; mean yield 1.4 kg/m<sup>2</sup>), while it emerged that drip irrigation can be useful in reducing BDM (−23.1% BDM severity). Therefore, this study suggests that the crop protection strategies tested, even if not definitive solutions, can significantly contribute to manage BDM more effectively, while preserving basil productivity and quality.

**Keywords** *Peronospora belbahrii* · Basil variety · Irrigation system · *Ocimum basilicum*

## Introduction

*Ocimum* L. is the major genus of herbs in the family *Lamiaceae*, with more than 150 species (Chowdhury et al. 2017). The term *basil* comprises numerous plants belonging to this

genus (Singletary 2018) and, among them, sweet basil (*O. basilicum* L.) is the most relevant from an economic point of view in the Western hemisphere (Pyne et al. 2014).

Basil is an important crop in Italy and the interest is increasing in different areas, with about 28.000 ha: approximately 70% in the north, 10% in the center and 20% in the south. About 1.000 ha of basil are grown in open field (Emilia-Romagna area covers about 30–40%), with prospects of future increase. A very interesting area for Italian basil cultivation is Liguria, famous for its “Basilico Genovese”, a PDO (Protected Designation of Origin) protected by a consortium.

Basil represents a highly specialised crop, which offers the possibility of at least 3–4 harvests/year. Its cultivation is particularly sensible to climate conditions: basil growing cycle in open field needs mild season which make cultivation possible from May to September in temperate zone.

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Therefore, growing a healthy crop managed with proper cropping system and suitable inputs is crucial.

Nowadays, Basil Downy Mildew (BDM) is the foremost disease which affect the crop, causing huge losses (Falach-Block et al. 2019). Since its first report in Uganda in 1932, the problem has been signalled in different countries throughout the world, including Italy (Garibaldi et al. 2004a). Basil leaf blades are commonly infected by *Peronospora belbahrii*, an obligate *Oomycete* that can also be present on other organs of the plant, including petioles and stems (Elad et al. 2016). Seeds were also found to be contaminated and constitute a potential source of inoculum (Garibaldi et al. 2004b).

*Peronospora belbahrii* infection cycle and ecological needs are only partially known; nevertheless, it is considered to commonly spreads asexually, while only little data regarding its sexual life cycle are available (Cohen et al. 2013b); since it is an obligate pathogen, it needs a host to survive (Johnson et al. 2022). Initial symptoms of the disease typically start with a yellowing of the adaxial surface of basil leaf tissues, usually in the mid-rib area, with yellowed areas usually bordered by leaf veins. Gradually, large chlorotic lesions with soft margins develop, that turn necrotic, leading to a slight curvature of leaves. Moreover, if environmental conditions are favourable, leaf wetness and 15–20 °C temperature, sporangiophores and sporangia, with a characteristic fuzzy, dark grey to purple growth, can be observed mainly on the abaxial leaf surface (Falach-Block et al. 2019; Zhang et al. 2019; Standish et al. 2020; Topolovec-Pintaric and Martinko 2020; Abdullah et al. 2022).

In recent years, some new strategies have been studied to control the disease, including chemical, physical, and genetic approaches (Cohen et al. 2017).

Based on literature reports, certain agricultural practices which generate adverse conditions for the pathogen are useful for disease control. For instance, providing good soil drainage and good air circulation among basil plants, maximizing plant spacing and using drip irrigation may inhibit the pathogen's growth. These techniques minimize leaf wetness and humidity that are crucial both for infection and for sporulation (Topolovec-Pintaric and Martinko 2020), since it is known that BDM spreads quickly through plantings especially when humidity is high, temperature is mild, air circulation is low, and periods of leaf wetness are extended (Wyenandt et al. 2010).

Some useful chemicals against BDM are available, including products containing mefenoxam, azoxystrobin, mandipropamid, fenamidone, oxathiapiprolin, phosphorus acids and cyazofamid; however, resistant populations are developing and some compounds that were previously efficient are now losing effectiveness (Gilardi et al. 2013; Cohen et al. 2017). Mefenoxam, for instance, was found to be one of the highly effective products against BDM,

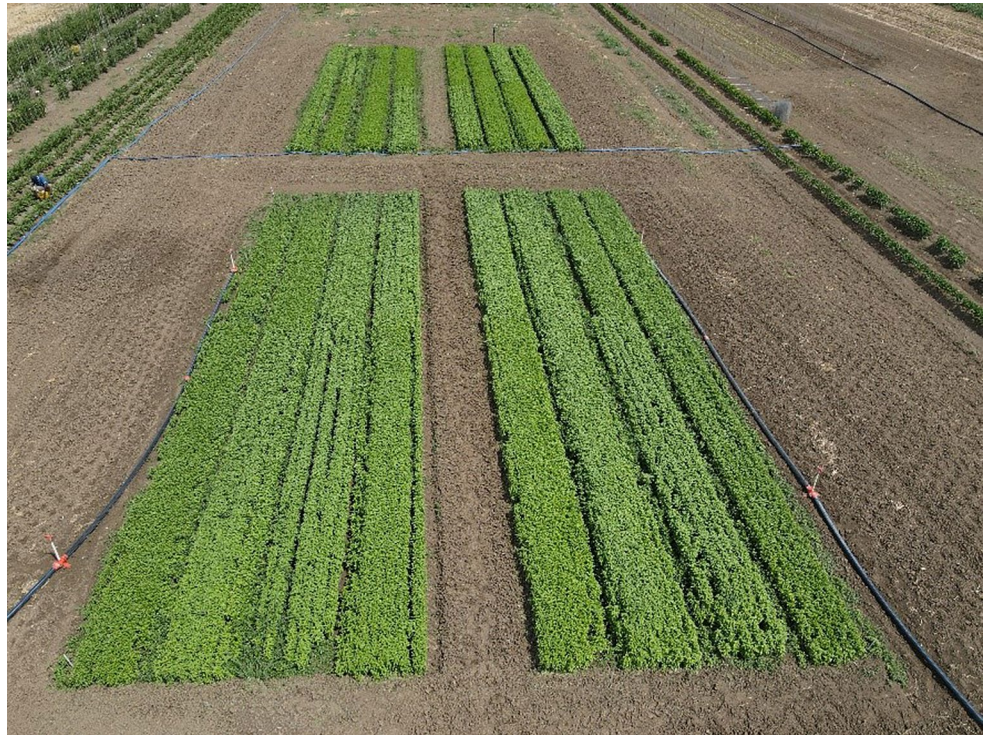
but mefenoxam-resistant isolates of *P. belbahrii* have been identified, making it ineffective; in the same way, other compounds are losing their efficacy over time (Cohen et al. 2017). In organic farming, chemical fungicides are not allowed, and also the green deal put them as very last tool, just when all the other alternatives were considered without solving the issue. Therefore, solutions listed by the Organic Materials Review Institute (OMRI) should be evaluated, like neem oil, potassium bicarbonate, hydrogen dioxide and some bio-products based on *Bacillus amyloliquefaciens*, *Streptomyces lydicus* and the extract of *Reynoutria sachalinensis* (Wyenandt et al. 2015; Topolovec-Pintaric and Martinko 2020).

As regards physical means, Cohen et al. (2017) reported the effectiveness of basil nocturnal illumination, since sporulation in darkness is much higher than under light (Cohen et al. 2013a; López-López et al. 2014). Cohen and Rubin (2015) affirmed that daytime solar heating is an interesting strategy to control BDM, while Cohen and Ben-Naim (2016) stated that nocturnal fanning reduced dew deposition on basil leaves, preventing both infection and sporulation of *P. belbahrii*. These practices cannot be applied in field but could help in greenhouse cultivation. Another interesting possibility to manage the disease is ozone (O<sub>3</sub>), in both gaseous and aqueous phases. Due to its strong oxidant power, O<sub>3</sub> is in fact widely used to control fungal growth and for mycotoxin detoxification, since it is effective and does not leave residues, as revealed by studies conducted on muskmelon fruits, wheat and dried figs (Wu et al. 2006; Zorlugenç et al. 2008; Hua-Li et al. 2018). The efficacy of this practice is demonstrated in controlled environments, technology not ready for application in open field in experimental plots.

Finally, genetic resistance was demonstrated as effective by several studies (Wyenandt et al. 2010; Farahani-Kofoet et al. 2014; Pyne et al. 2014). According to Homa et al. (2016), *Ocimum* species and cultivars with the greatest downward leaf curvature and the highest stomatal densities are more susceptible. Therefore, since cultivars have a different susceptibility to the pathogen, the selection of resistant basils is crucial to lower losses, and the transfer of genetic resistance to sweet basil is under way (Cohen et al. 2017).

The overall objective of this study was to optimise basil productivity and quality and limit BDM occurrence in organic farming. Based on data available in literature, a two-year study was organised including: two basil varieties with different susceptibility to BDM, two different sowing densities, and two irrigation systems, drip and sprinkler.

Fig. 1 Experimental field



## Materials and methods

### Experimental trial

The study was held in Stuard Experimental Farm (*Azienda Agraria Sperimentale Stuard*) in Parma, Italy, in the agricultural years 2021 and 2022 following organic farming practices, with techniques commonly adopted by expert farmers for basil production for local industries. The geographical coordinates of the experimental areas were 44.807232°N and 10.271823°E in 2021, and 44.807206°N and 10.273990°E in 2022.

For both years, the land was subjected to ploughing, harrowing, division into parcels, rolling, and a second harrowing to facilitate the elimination of weeds. Sowing was preceded by fertilization with NPK organic mineral fertilizer containing magnesium and sulphur (Tiger NPK 3-6-12, Fomet, IT; 2000 kg/ha) and an organic nitrogen fertilizer (Dermazoto N8, Organozoto, IT; 1000 kg/ha), using a drag harrow to facilitate the burying of the fertilizer followed by a fixed-teeth harrow to eliminate any residual weed.

Two varieties of basil were tested, one considered resistant to BDM (variety 1, resistant) and the other more susceptible to the pathogen (variety 2, medium susceptible), adopting two sowing densities:

- Dense, a dose of 30 kg/ha in 16 rows per single bed planting (width 1.5 m and row distance 8 cm);

**Table 1** Date of the sowing and of the fourth basil cuts in the 2 years of the trial (2021 and 2022)

| Operations | Years |      |
|------------|-------|------|
|            | 2021  | 2022 |
| Sowing     | 28/5  | 3/6  |
| First cut  | 16/7  | 22/7 |
| Second cut | 10/8  | 11/8 |
| Third cut  | 3/9   | 5/9  |
| Fourth cut | 24/9  | 29/9 |

- Sparse, using a dose of 15 kg/ha in 8 rows per single bed planting (width 1.5 m and row distance 16 cm).

The experimental protocol also included two irrigation modes, sprinkler (Akplas; 5 mm/h) and drip (Plastic-Puglia, Aquatape; 1 L/h/dripper), with irrigation treatments carried out twice a week, from June to September, for a total irrigation volume of 435 mm and 492 mm in 2021 and 2022, respectively.

Therefore, overall, the thesis compared were 8 (= 2 basil varieties\* 2 irrigation systems\* 2 sowing densities). Trials were carried out with 4 replicates. For both experimental years, the field scheme included 32 experimental plots (=8 thesis\* 4 replicates) measuring 15 m<sup>2</sup> (Fig. 1).

Meteorological data were collected using a data logger. The parameters measured were: air temperature (°C) and rainfall (mm).

Relevant time schedules were summarized in Table 1.



## Crop management

The agronomic practices were slightly adapted depending on the crop year and the trend of climatic conditions.

Fertilizers and disinfectant products applied in field in the 2 years of the study are summarized in Table 2. These products were applied with different frequencies after sowing and after each cut of basil. In the first year of the study, Amylo-X® was applied every 3 days for the whole crop season (from June to September); after each cut, instead, Fito Bacter® was applied 2 times every 2 days. In 2022, Amylo-X® was no more employed, while Fito Bacter® was applied every 3 days. The fertilizer units used were 320 kg/ha of N, 120 kg/ha of P<sub>2</sub>O<sub>5</sub>, and 240 kg/ha of K<sub>2</sub>O.

Regarding the irrigation, in 2021, the field was irrigated twice a week, applying a different volume of water depending on the climatic conditions (6 mm in June, 24 mm in July, 15–18 mm in August and 6–12 mm in September), for a total irrigation volume of 435 mm. In 2022, the field was irrigated twice a week, using a higher irrigation volume than the previous year due to the exceptional climatic conditions and particularly hot climate (10–18 mm in June, 24–30 mm in July, 18 mm in August and 12–18 mm in September), for a total irrigation volume of 492 mm.

## Sampling

Over 2 years of cultivation 4 samplings were done, before each cut performed on the basil. Evaluations were made concerning both agronomic parameters and BDM incidence.

**Table 2** Fertilizers and disinfectant products used in the trial; products dosage and companies are reported

| Commercial product  | Active ingredient  | Company                      | Dosage applied |
|---------------------|--|------------------------------|----------------|
| Idrox® 20           | Copper hydroxide (20%)   | Manica Spa, IT               | 2 kg/ha        |
| Fito Bacter®        | Zinc (2%)  | Fitokem, IT                  | 2 kg/ha        |
| Borlanda Fluida NKC | Organic nitrogen (3%)  | Ecovigor AA, Timac Agro, IT  | 6 L/ha         |
| Amylo-X®            | <i>Bacillus amyloliquefaciens</i> , strain D747 (5*10 <sup>10</sup> CFU/g) | Biogard, IT                  | 2.1 kg/ha      |
| Siapton®            | Amino acids and proteins   | Sumitomo Chemical Italia, IT | 1.3 L/ha       |
| Megafol®            | Vitamins, amino acids and proteins   | Valagro, IT                  | 3 L/ha         |
| Trainer®            | Amino acids and peptides   | Hello Nature, IT             | 5 L/ha         |
| Fertil 10           | Organic nitrogen (10%)   | ILSA Spa, IT                 | 666 kg/ha      |
| Dermazoto N11       | Organic nitrogen (8%)  | Organazoto, IT               | 500 kg/ha      |

Sampling was carried out in 3 test areas of 0.09 m<sup>2</sup> for each thesis with a proven and specific methodology for each agronomic parameters (unpublished data).

The agronomic parameters measured for each sampling area were the following:

- Plant height (cm): average height of basil plants;
- Flowering Index (FI): number of plants with inflorescences;
- Yield: weight (kg) of basil, subsequently referred to 1 m<sup>2</sup>;
- Density: number of plants, subsequently referred to 1 m<sup>2</sup>.

Furthermore, in the sampling area, 10 plants were randomly collected to measure two agronomic parameters:

- Woodiness Index (WI), qualitative index based on stem consistency assessment with a scale from 0 (soft stem) to 5 (woody stem);
- Leaves (% by weight), that represent the ratio between the weight of the leaves and the stem.

As regards BDM assessment, 10 plants were randomly sampled for each replicate of each thesis (all 32 plots), and the disease incidence (%) was calculated as number of plants showing even mild symptoms/10 plants. Moreover, in symptomatic plants, the BDM severity was also computed, as number of leaves with symptoms/total leaves, according to McGrath (2020).

## Statistical analysis

Leaves (%), disease incidence (%) and disease severity (%) were arcsin transformed to homogenize means (Clewer and Scarisbrick 2001). All data were subjected to one way analysis of variance (ANOVA) and Tukey's test was used to compare means. The statistical package PASW statistics was used for data analysis (ver. 27, SPSS Inc., Chicago, USA, 2021).

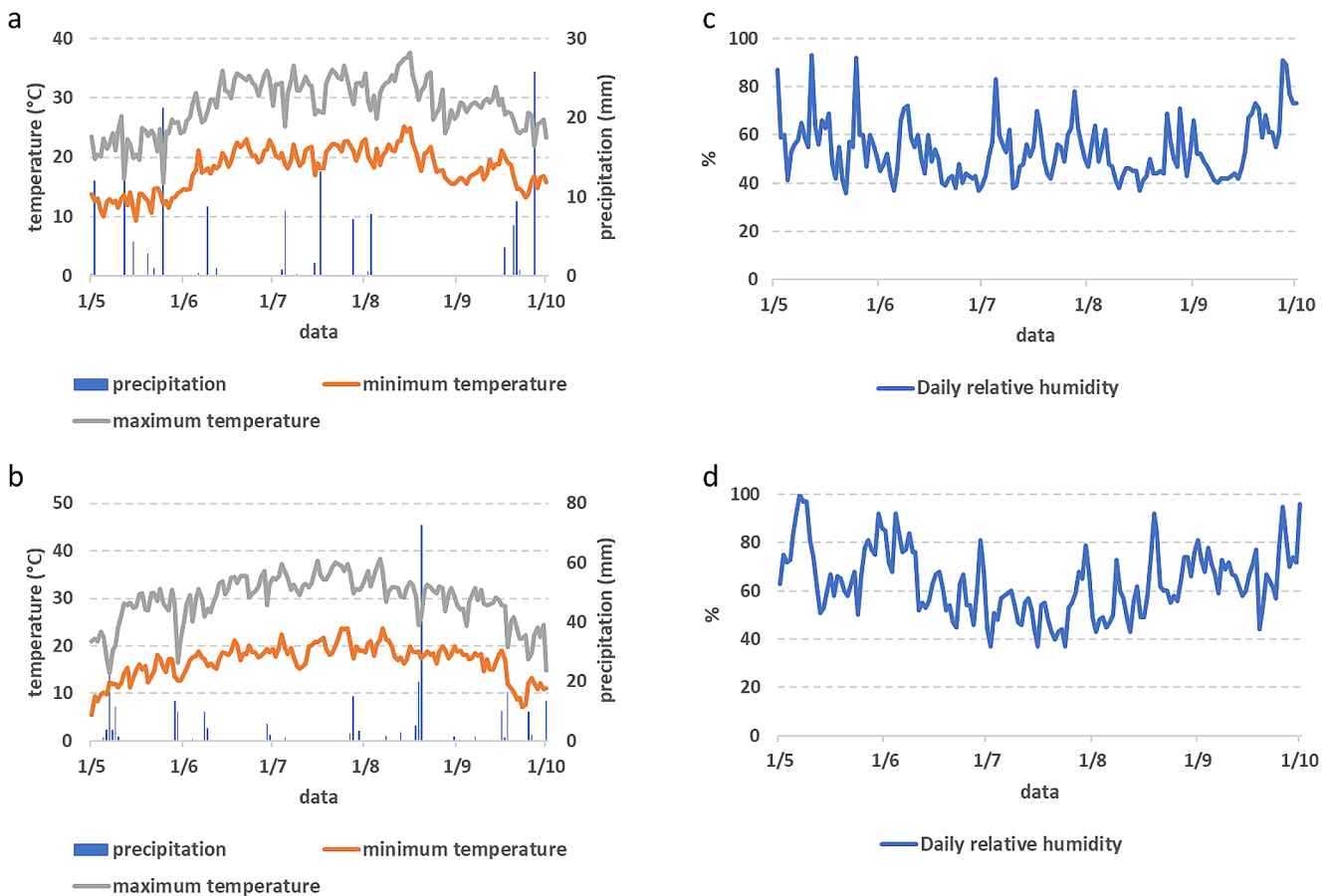
Pearson correlation coefficients between BDM and agronomic parameters were also computed.

## Results

### Meteorological data

Meteorological data collected during the trial in 2021 and 2022 are reported in Fig. 2.

In 2021, below average temperatures occurred from mid-April to late May, causing problems and delays in the



**Fig. 2** Meteorological data (minimum and maximum temperatures, °C, daily precipitation, mm, and daily relative humidity, %) collected between May and October 2021 (a, c) and 2022 (b, d) in Parma, Italy (Parma and San Pancrazio Station, respectively)

emergency and early growth stages of basil crop. In fact, in the second half of April, minimum temperatures were much lower than 10 °C. In June and July, maximum temperatures rarely exceeded 35 °C, with temperature ranges from 14.5 to 34.5 °C and from 17 to 35.4 °C in June and July, respectively and dry weather (about 40 mm of precipitation from early June to late July).

In 2022, both spring and summer were characterized by low rainfall and maximum temperatures above average (well above 35 °C for all summer months, with peaks of up to 40 °C in early July). During the period considered (from early April to late September), precipitation was accumulated by about 300 mm, abnormalities mainly concerning the first months of the year (about 50 mm of precipitation between January and March 2022; data not shown). In mid-September, there was a sharp drop in temperatures (especially the minimum, that were much lower than 10 °C), which caused delay in crop growth and the impossibility of making the last cut, that is generally performed in late-September.

### Agronomic parameters

Due to very different meteorological conditions in the 2 years of the study, the agronomic variables showed significant difference. In 2022, plant height, FI and yield/m<sup>2</sup> were lower than in 2021 (mean reduction of 12%, 58% and 41%, respectively;  $p < 0.01$ ; Table 3). On the contrary, WI was significantly higher and almost doubled in 2022 compared to 2021 ( $p < 0.01$ ; Table 3).

Moreover, differences were detected in the four samplings for all the variables considered. In fact, during the growing season, from the first sampling to the last one, plant height and WI increased (+20% and +58%, respectively;  $p < 0.01$ ), while FI, basil yield, density, and leaves % decreased (mean reduction of 46%, 29%, 35% and 13%, respectively;  $p < 0.01$ ).

As regards the two irrigation systems, drip and sprinkler, differences were detected in yield/m<sup>2</sup> (1.5 vs 1.3 kg/m<sup>2</sup>, respectively;  $p < 0.05$ ), plant density (308.9 vs 363.5 plants/m<sup>2</sup>, respectively;  $p < 0.01$ ) and WI (41.1 vs 50.3, respectively;  $p < 0.01$ ).

**Table 3** Analysis of variance of plant height (cm), Flowering Index (FI), yield (kg/m<sup>2</sup>), density (number of plants/m<sup>2</sup>), woodiness index (WI), leaves (% by weight), BDM incidence (%), and BDM severity (%) in the 4 samplings in the 2 years of the study (2021 and 2022), with the 2 irrigation systems (drip and sprinkler) and the 2 sowing densities (dense and sparse), for the 2 basil varieties evaluated (variety 1, considered resistant, and variety 2, considered more susceptible to BDM)

|                   | Plant height (cm) | FI <sup>§</sup>   | Yield (kg/m <sup>2</sup> ) | Density (plants/m <sup>2</sup> ) | WI <sup>§</sup>   | Leaves (%)        | BDM <sup>§</sup> incidence (%) | BDM severity (%)  |
|-------------------|-------------------|-------------------|----------------------------|----------------------------------|-------------------|-------------------|--------------------------------|-------------------|
| A. Year           | **                | **                | **                         | n.s.                             | **                | n.s.              | **                             | **                |
| 2021              | 45.9 <sup>a</sup> | 10.2 <sup>a</sup> | 1.7 <sup>a</sup>           | 341.9                            | 34.2 <sup>b</sup> | 65.8              | 37.4 <sup>b</sup>              | 13.3 <sup>b</sup> |
| 2022              | 40.4 <sup>b</sup> | 4.3 <sup>b</sup>  | 1.1 <sup>b</sup>           | 330.4                            | 57.2 <sup>a</sup> | 67.3              | 79.5 <sup>a</sup>              | 39.9 <sup>a</sup> |
| B. Sampling       | **                | **                | **                         | **                               | **                | **                | **                             | **                |
| 1                 | 37.6 <sup>a</sup> | 9.8 <sup>b</sup>  | 1.7 <sup>b</sup>           | 363.3 <sup>bc</sup>              | 26.0 <sup>a</sup> | 68.6 <sup>b</sup> | 10.5 <sup>a</sup>              | 2.4 <sup>a</sup>  |
| 2                 | 45.5 <sup>b</sup> | 7.1 <sup>a</sup>  | 1.6 <sup>b</sup>           | 400.2 <sup>c</sup>               | 47.4 <sup>b</sup> | 68.5 <sup>b</sup> | 73.4 <sup>b</sup>              | 33.4 <sup>b</sup> |
| 3                 | 44.5 <sup>b</sup> | 6.7 <sup>a</sup>  | 1.1 <sup>a</sup>           | 344.6 <sup>b</sup>               | 47.6 <sup>b</sup> | 69.2 <sup>b</sup> | 74.8 <sup>b</sup>              | 31.5 <sup>b</sup> |
| 4                 | 44.9 <sup>b</sup> | 5.3 <sup>a</sup>  | 1.2 <sup>a</sup>           | 236.6 <sup>a</sup>               | 61.8 <sup>c</sup> | 60.0 <sup>a</sup> | 75.0 <sup>b</sup>              | 38.9 <sup>c</sup> |
| C. Irrigation     | n.s.              | n.s.              | *                          | **                               | **                | n.s.              | n.s.                           | *                 |
| Drip              | 43.4              | 7.2               | 1.5 <sup>a</sup>           | 308.9 <sup>b</sup>               | 41.1 <sup>b</sup> | 67.2              | 57.5                           | 23.8 <sup>b</sup> |
| Sprinkler         | 42.8              | 7.23              | 1.3 <sup>b</sup>           | 363.5 <sup>a</sup>               | 50.3 <sup>a</sup> | 66.0              | 59.4                           | 29.3 <sup>a</sup> |
| D. Variety        | **                | **                | **                         | **                               | n.s.              | n.s.              | **                             | **                |
| 1                 | 44.7 <sup>a</sup> | 1.3 <sup>b</sup>  | 1.5 <sup>a</sup>           | 377.3 <sup>b</sup>               | 44.6              | 66.4              | 40.2 <sup>b</sup>              | 21.3 <sup>b</sup> |
| 2                 | 41.6 <sup>b</sup> | 13.1 <sup>a</sup> | 1.3 <sup>b</sup>           | 295.0 <sup>a</sup>               | 46.8              | 66.7              | 76.6 <sup>a</sup>              | 31.8 <sup>a</sup> |
| E. Sowing density | *                 | n.s.              | n.s.                       | **                               | n.s.              | n.s.              | n.s.                           | n.s.              |
| Dense             | 42.1 <sup>b</sup> | 6.6               | 1.4                        | 374.0 <sup>a</sup>               | 43.2              | 65.9              | 58.8                           | 26.7              |
| Sparse            | 44.1 <sup>a</sup> | 7.8               | 1.4                        | 298.4 <sup>b</sup>               | 48.2              | 67.3              | 58.1                           | 26.5              |
| Interaction       |                   |                   |                            |                                  |                   |                   |                                |                   |
| A*B               | **                | **                | **                         | **                               | **                | *                 | **                             | **                |
| A*C               | *                 | n.s.              | **                         | **                               | **                | n.s.              | n.s.                           | n.s.              |
| A*D               | **                | **                | **                         | n.s.                             | n.s.              | n.s.              | **                             | **                |
| B*C               | **                | n.s.              | **                         | **                               | **                | **                | n.s.                           | n.s.              |
| B*D               | **                | **                | n.s.                       | n.s.                             | n.s.              | *                 | **                             | **                |

Only significant interactions are reported. Different letters indicate significant differences according to Tukey's test ( $p < 0.01$ )

\*\* $p < 0.01$ , \* $p < 0.05$ , n.s. not significant

<sup>§</sup>FI Flowering Index, WI Woodiness Index, BDM Basil Downy Mildew

Comparing the two basil varieties, variety 1 was higher than variety 2 (44.7 vs 41.6 cm, +8%;  $p < 0.01$ ), and produced more (1.5 vs 1.3 kg/m<sup>2</sup>, +11%;  $p < 0.01$ ), with a higher investment (377 vs 295 plants/m<sup>2</sup>, +28%;  $p < 0.01$ ). On the contrary, variety 1 showed a lower FI, compared to variety 2 (−90%;  $p < 0.01$ ).

Considering the two sowing densities, dense and sparse, differences were detected in plant height (42.1 vs 44.1 cm;  $p < 0.05$ ), and plant investment/m<sup>2</sup> (374.0 vs 298.4 plants/m<sup>2</sup>;  $p < 0.01$ ).

Moreover, the interaction year × irrigation impacted significantly on plant height ( $p < 0.05$ ), yield ( $p < 0.01$ ), plant density ( $p < 0.01$ ), and WI ( $p < 0.01$ ; Fig. 3). For all the variables considered, the behavior was the opposite in 2021 and 2022. The interaction year × variety was significant too, considering plant height ( $p < 0.01$ ), FI ( $p < 0.01$ ), and yield ( $p < 0.01$ ; Fig. 4). As a general comment, the difference between the 2 varieties was very limited/absent in 2022. Finally, also the interaction sampling × year for all the variables considered was significant ( $p < 0.01$ ; Fig. 5),

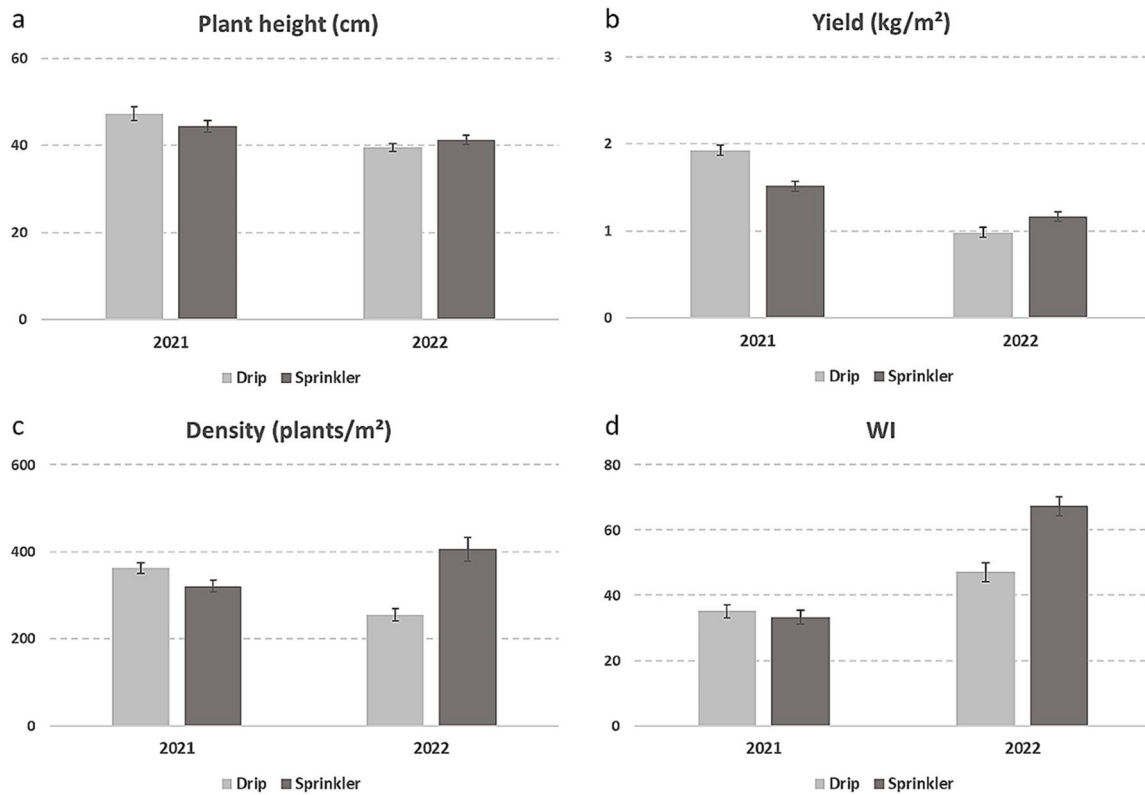
as well as sampling × irrigation, considering all the variables except FI ( $p < 0.01$ ; Fig. 6), and sampling × variety, considering plant height ( $p < 0.01$ ), FI ( $p < 0.01$ ) and leaves % ( $p < 0.05$ ; Fig. 7).

### BDM incidence and severity

*P. belbahrii* was more effective in 2022 than in 2021 in basil plant attack. Indeed, the average BDM incidence in 2022 and 2021 was 79.5% and 37.4%, respectively ( $p < 0.01$ ), while the average severity was 39.9% and 13.3%, respectively (Table 3;  $p < 0.01$ ).

Differences were detected in the 4 samplings, considering both the disease incidence (10.5% vs 75.0% in the first and last sampling;  $p < 0.01$ ), and the disease severity (from 2.4% vs 38.9%;  $p < 0.01$ ).

As regards the two varieties, variety 2 (considered medium susceptible to BDM) presented a higher BDM incidence and severity than variety 1 (considered resistant to BDM), (76.6% vs 40.2%, and 31.8% vs 21.4%, respectively;



**Fig. 3** Plant height (cm; **a**), yield (kg/m<sup>2</sup>; **b**), density (number of plants/m<sup>2</sup>; **c**), and Woodiness Index (WI= stem consistency assessment, 0–5 scale; **d**) of basil plants irrigated by drip or sprinkler, in the 2 years of the study (2021 and 2022)

$p < 0.01$ ). Nevertheless, the interaction between year and variety was significant, considering both disease incidence (Fig. 8, a;  $p < 0.01$ ) and severity (Fig. 8, b;  $p < 0.01$ ). In fact, in 2021, variety 1 did not show any BDM symptom; however, in 2022, this variety was affected by *P. belbahrii* too, with incidence and severity comparable to variety 2.

The irrigation systems significantly impacted on BDM severity. In fact, in plants irrigated with the sprinkler system, the disease severity was higher than in those irrigated with the drip one (29.3% vs 23.8%, respectively;  $p < 0.05$ ). No effect of sowing densities was observed.

Finally, the interaction sampling  $\times$  year (Fig. 9) and sampling  $\times$  variety (Fig. 10) were significant too, both for disease severity ( $p < 0.01$ ) and disease incidence ( $p < 0.01$ ).

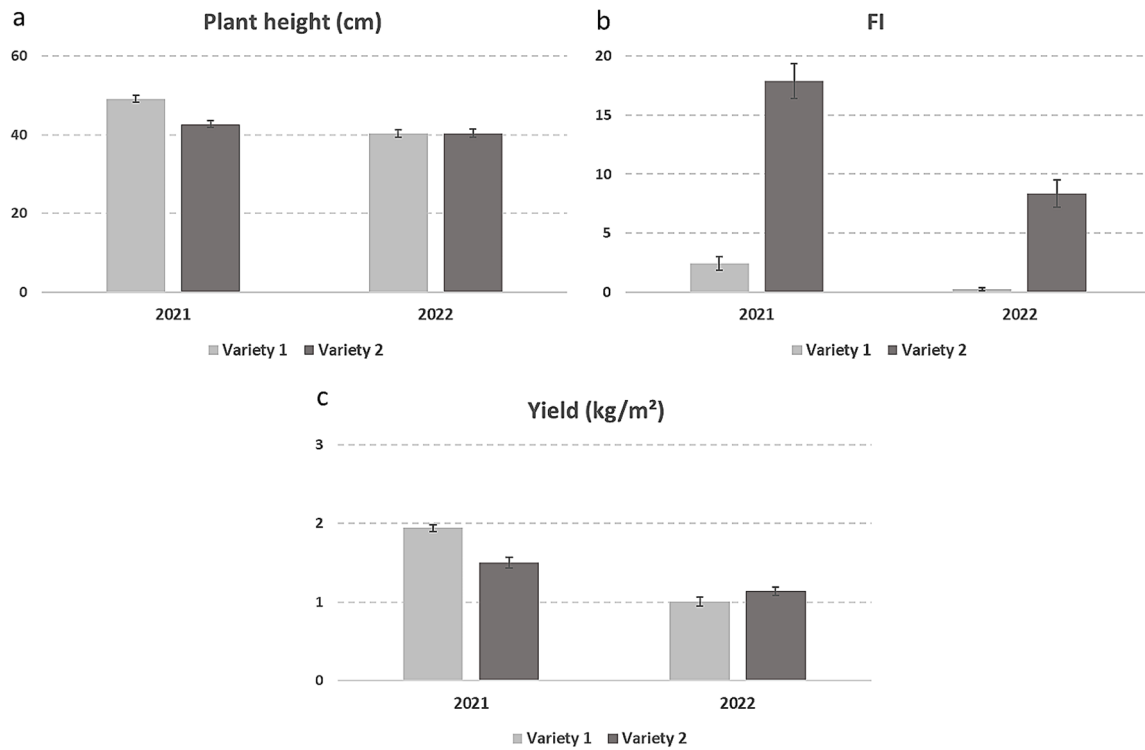
Pearson correlation was run for BDM and all the agronomic factors and a positive correlation between disease severity and WI ( $r = 0.61$ ) was observed, as well as a negative correlation between yield and BDM incidence ( $r = -0.62$ ) and severity ( $r = -0.60$ ).

## Discussion

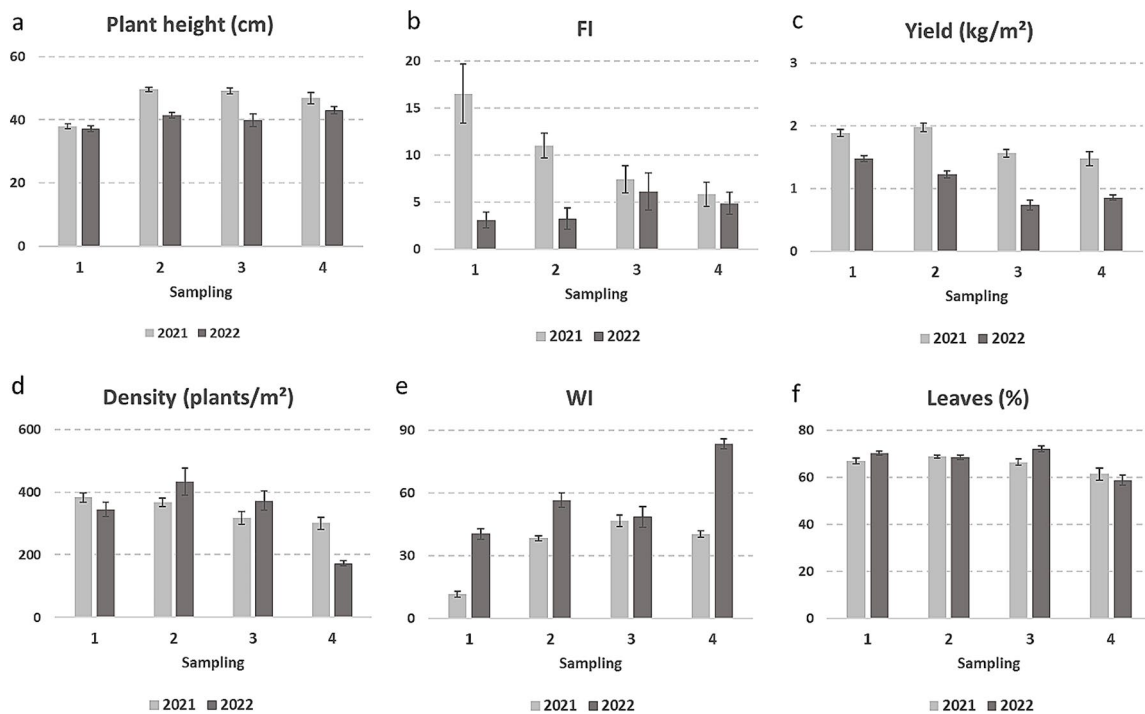
BDM caused by the oomycete *P. belbahrii* has recently become one of the most devastating diseases of basil worldwide (Gunacti 2023), causing severe damages and crop losses, with a significant economic impact (Abdullah et al. 2022).

Currently, BDM is mainly controlled by fungicide applications (Mersha et al. 2012), but the development of resistances to chemical products is supporting the search for alternative practices that may help to prevent the disease, minimizing, at the same time, chemical residues at harvest (Omer et al. 2021). In addition, there is a strong attention to consumer health and many attempts are ongoing to reach zero residue or offer organic products to consumers.

Therefore, the present study, conducted in two consecutive years (2021 and 2022) in Northern Italy in organic farming conditions, aimed to combine agronomic strategies for basil protection against BDM. These included testing the role of plant investment, resistant varieties, as well as the use of different irrigation methods. The choice to use agronomic means was linked to the fact that biological products, if applied alone, showed very limited effects against *P. belbahrii* in several studies (Gilardi et al. 2013; Zhang et

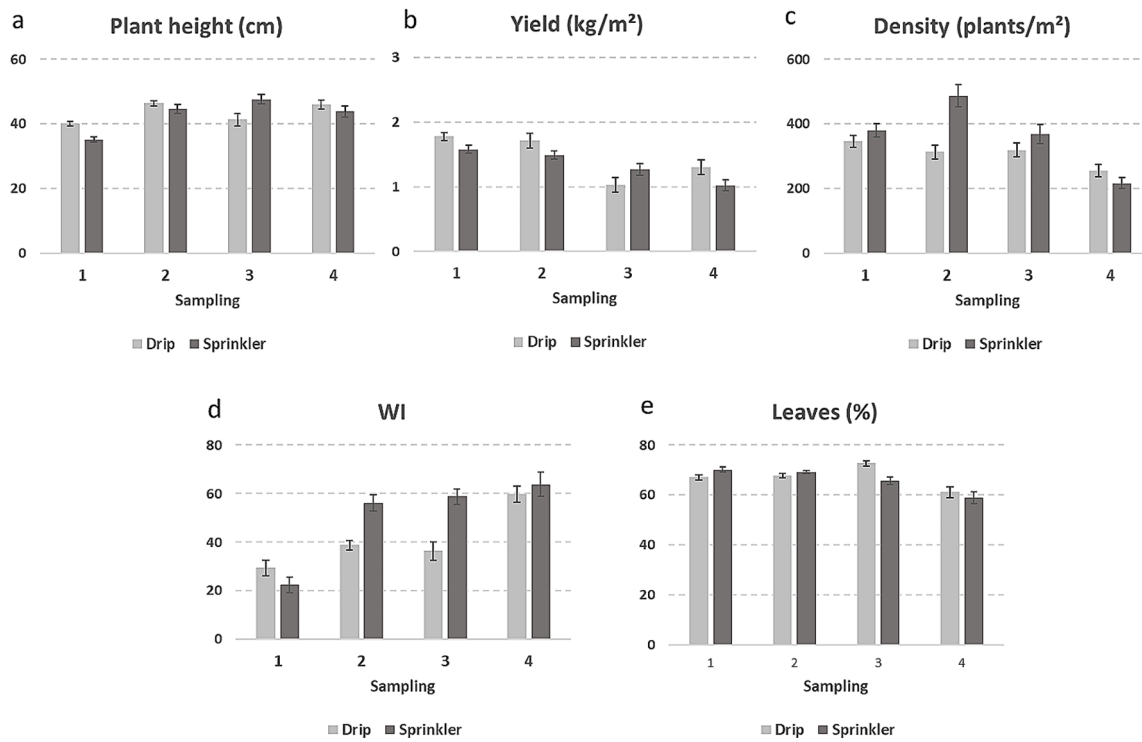


**Fig. 4** Plant height (cm; **a**), Flowering Index (FI=number of plants with inflorescences; **b**) and yield (kg/m<sup>2</sup>; **c**) of variety 1 (considered resistant) and variety 2 (considered medium susceptible to BDM) in the 2 years of the study (2021 and 2022)

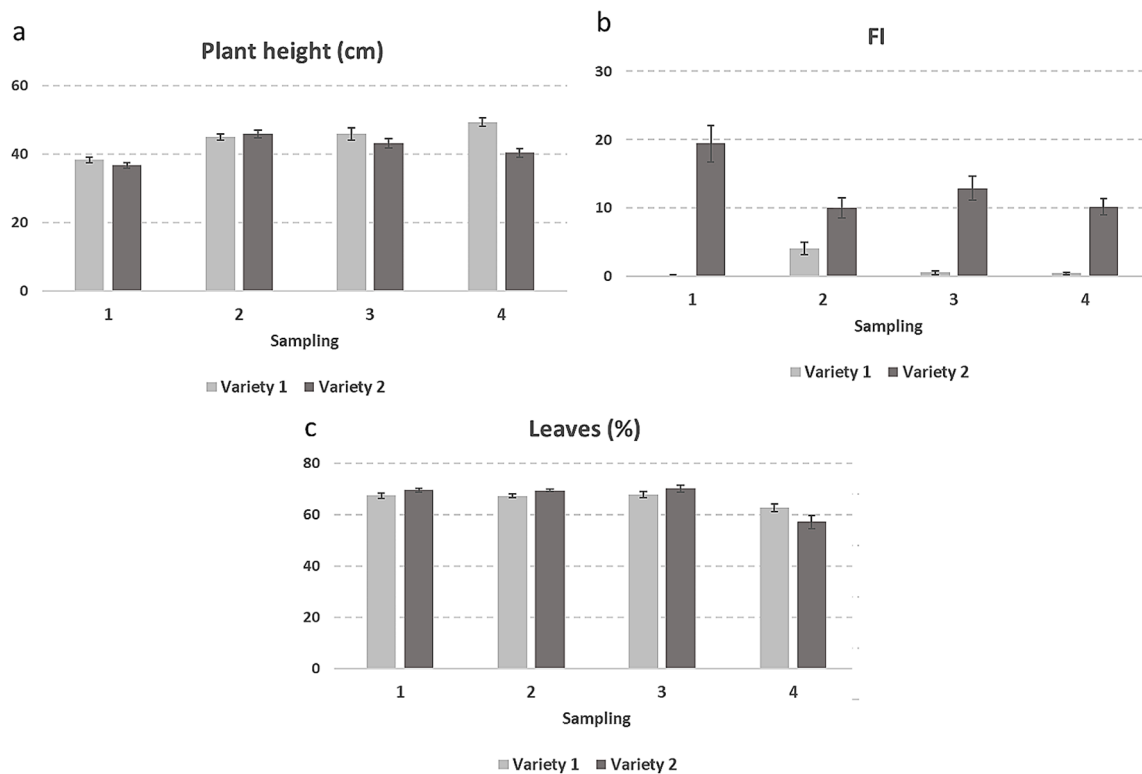


**Fig. 5** Plant height (cm; **a**), Flowering Index (FI=number of plants with inflorescences; **b**), yield (kg/m<sup>2</sup>; **c**), density (plants/m<sup>2</sup>; **d**), woodiness Index (WI; **e**) and leaves (%; **f**) in the 4 samplings in the 2 years of the study (2021 and 2022)

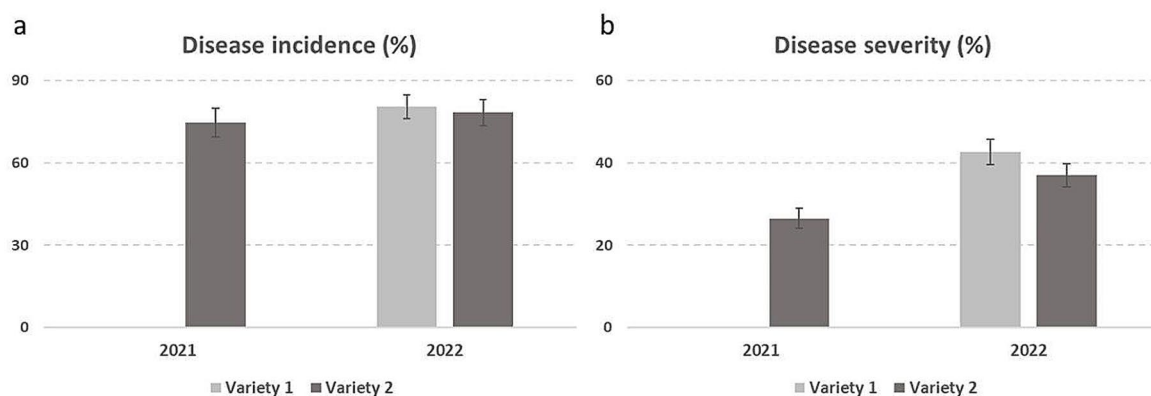




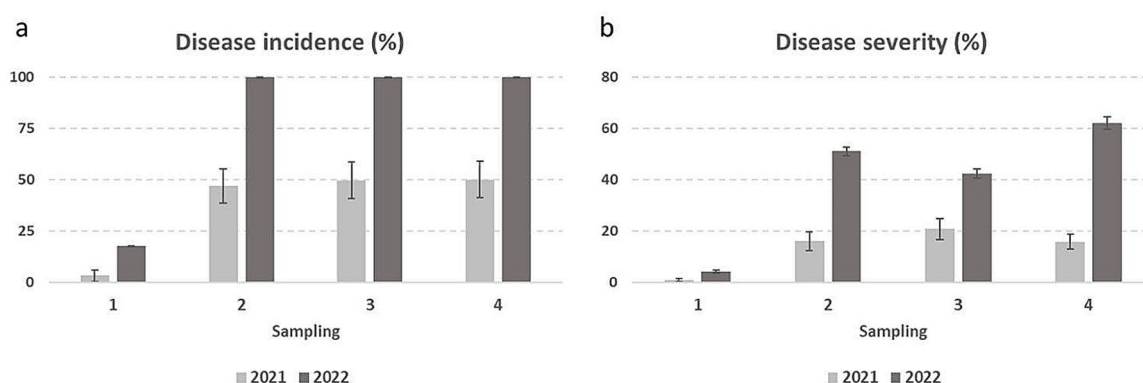
**Fig. 6** Plant height (cm; **a**), yield (kg/m<sup>2</sup>; **b**), density (plants/m<sup>2</sup>; **c**), woodiness Index (WI=stem consistency assessment, 0–5 scale; **d**) and leaves (leaves= ratio between the weight of the leaves and the stem, %; **e**) of basil plants irrigated by drip or sprinkler, in the 4 sampling times



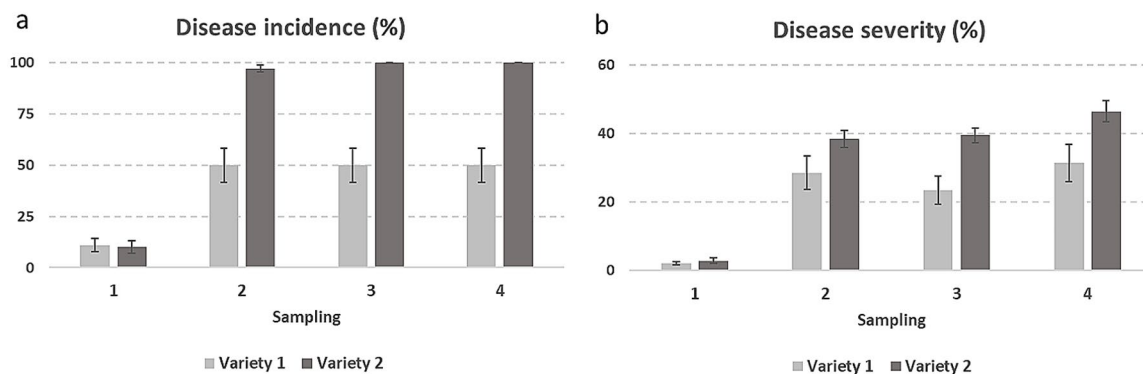
**Fig. 7** Plant height (cm; **a**), Flowering Index (FI=number of plants with inflorescences; **b**) and leaves (leaves= ratio between the weight of the leaves and the stem, %; **c**) of variety 1 (considered resistant) and variety 2 (considered medium susceptible to BDM) in the 4 sampling times



**Fig. 8** Disease incidence (%; **a**) and disease severity (%; **b**) of variety 1 (considered resistant) and variety 2 (considered more susceptible to BDM) in the 2 years of the study (2021 and 2022)



**Fig. 9** Disease incidence (%; **a**) and disease severity (%; **b**) in the 4 samplings, in the 2 years of the study (2021 and 2022)



**Fig. 10** Disease incidence (%; **a**) and disease severity (%; **b**) of variety 1 (considered resistant) and variety 2 (considered more susceptible to BDM) in the 4 sampling times

al. 2019); this was also confirmed by the current work, in which both Fito Bacter® and Amilo-X® were not effective against BDM.

BDM was most severe in 2022, mainly due to the different climatic conditions that occurred in the two years. Indeed, it is known that epidemics of *P. belbahrii* largely

depend upon climate conditions (Abdullah et al. 2022), and the effects of temperature and relative humidity have been confirmed (Omer et al. 2021). Summer of 2021 was characterized by dry weather and maximum temperatures that rarely exceeded 35°C, favorable conditions for the development of basil, but apparently not conducive for

BDM development. On the contrary, in 2022, particularly high temperatures and repeated drought phenomena caused serious agronomic management problems to the basil crop, with high water needs. This led to a faster aging of plants, a reduction in terms of product quality, basil stress and BDM severe outbreak. These results are apparently not in agreement with data reported by Cohen et al. (2017), who reported a limiting effect of temperature higher than 35 °C on different steps of *P. belbahrii* infection cycle. Also yield resulted lower in 2022 than 2021 for both varieties, with a confirmed correlation with BDM incidence and severity.

Irrigation plays an important part in managing plant diseases. Drip irrigation, in addition to a more efficient use of water, avoids wetting of aerial plant parts and generally results in lower incidence and severity of leaf diseases (Manda et al. 2021; Rhouma et al. 2022). In the present study, drip irrigation significantly reduced disease severity, compared to sprinkler, in agreement with other reports, without significant effect on basil production in qualitative and quantitative terms. In spinach, for instance, downy mildew incidence was lower in plots drip irrigated when compared to sprinkler (Montazar et al. 2019). Similarly, Wyenandt et al. (2015) stated that drip irrigation, instead of bare ground and overhead irrigation, could be useful in reducing BDM, especially in zones where disease pressure is lower.

Two basil varieties were included in the study because of their different reported susceptibility to the *P. belbahrii* (Johnson et al. 2022). In 2021, the results confirmed what was expected; variety 1 (considered resistant to BDM) indeed showed no BDM symptoms, while variety 2 (medium susceptible) was affected by the pathogen. However, in the second year (2022), variety 1 and 2 showed comparable BDM incidence and severity. A wide discussion occurred regarding possible explanation of this unexpected event, confirmed in many open field basil crops grown in north Italy (Basil chain stakeholders, advisory board). Meteorological conditions that occurred in the 2 years, specifically the exceptional high temperatures registered in 2022, most probably played a main role. In fact, it is known that unusual temperature can cause changes in host-pathogen interactions, and, at the same time, can also facilitate the emergence of new races of the pathogen, which in turn break down host-plant resistance (Hunjan and Lore 2020). In this regard Ghorbani (2007) stated that resistance can be race-specific and growing the same varieties year after year in an area can led to evolution of new races or more aggressive strains of a fungus and breakdown of resistance in the host crop. Thus, another possible explanation of what was observed in 2022 is that new strains of the pathogens developed, towards which variety 1 does not have resistance. Ben-Naim and Weitman (2022) also reported a new virulent race of *P. belbahrii* after the introduction of variety 1, which

made its resistance ineffective or incomplete in some locations, such as in New Jersey.

Lastly, a further aspect taken into consideration in the present study was sowing density. A lower crop density has been shown to have a significant impact on the success of Integrated Pest Management control measures on different crops. In fact, the density at which plants are grown affects the relative humidity of the leaf canopy and the rapidity at which leaves dry after a wetting period and may thus impact on the development of foliar and soilborne diseases in several pathosystems (Gilardi et al. 2020). Confirming this, Elad et al. (2015) reported that increased plant spacing helped suppress both white mould caused by *Sclerotinia sclerotiorum*, and gray mold, caused by *Botrytis cinerea* on basil. As regards downy mildew of rose, caused by *P. sparsa*, it was demonstrated that reducing the density of container-grown plants also reduced the progress of the disease, even if to a lesser extent than when compared with the effect of fungicide treatments (O'Neill et al. 2002). Finally, concerning BDM, Omer et al. (2021) concluded that disease severity was reduced by diluted planting density, without any effect on yield, and, similarly, Gilardi et al. (2020) stated that high crop density had a significant positive impact on BDM development. In contrast to these results, in the present work, no differences were observed among the two sowing densities applied. This is not surprising because all the agronomic parameters measured resulted comparable, except plant height, a bit higher with sparse sowing; no effect of sowing density was observed also on crop yield. A possible explanation is related to plant behaviour: independently on sowing density, leaves were increasingly dense with time, especially after the first cutting. Therefore, the different effect of sparse or dense sowing densities on the relative humidity of basil leaves is irrelevant during the growing season. However, it is known that a sparse sowing density negatively affects WI, causing it to increase.

In conclusion, the present study showed that, even if the strategies tested were not sufficient to completely solve the problem, they can significantly contribute to manage BDM more effectively, if correctly combined. In fact, sparse sowing density of basil varieties considered more resistant to the pathogen, combined with drip irrigation, could significantly help reduce the spread of the disease. However, further research is required to better understand *P. belbahrii* epidemiology and thus improve BDM management.

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**Data availability** Data are available on request from the authors.

## Declarations

**Ethical approval** The authors declare all ethical aspect are respected.

**Financial interest** The authors have no relevant financial or non-financial interests to disclose.

**Conflict of interest** The authors declare no conflict of interest.

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