BIOLOGICAL CONTROL OF *BOTRYTIS* GRAY MOULD AND *SCLEROTINIA* DROP IN LETTUCE

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ABSTRACT

Research was carried out to evaluate the effectiveness of the biological control of two most important fungal diseases of lettuce (*Lactuca sativa* L.): 1) Botrytis Gray Mould caused by *Botrytis cinerea* Pers. ex Fr.; 2) Sclerotinia Drop caused by two pathogenic fungi, *Sclerotinia sclerotiorum* (Lib.) De Bary and/or *Sclerotinia minor* Jagger. Biological control in lettuce was carried out: 1) using *Coniothyrium minitans* Campbell, an antagonist fungus that attacks and destroys sclerotia within the soil; 2) identifying lettuce genotypes showing less susceptibility or tolerance. The object of this research was to find control strategies to reduce chemical treatments. The use of resistant varieties is one of the most economical ways to control vegeTable diseases.

The lettuce genotypes showing in preliminary trials the best behaviour to the sclerotial diseases were compared in a randomized complete block experiment design and replicated four times. Observations were carried out from February up to April registering the number of diseased plants and yield. The pathogens were isolated on PDA medium and identified. The isolates grown onto PDA plates, after incubation for 6 weeks, allowed obtaining sclerotia that were the target of *C. minitans* in biological control trials. In laboratory, in controlled conditions, 27 small plots (30 cm in diameter each) with disinfected soil were performed. In 18 plots 9 sclerotia were inoculated (per plot, three of each fungus) and in 9 plots of them a suspension of the antagonist fungus was added. Subsequently, three lettuce varieties were transplanted. For each variety were compared: 1) untreated plots; 2) treated plots with sclerotia only; 3) treated plots with sclerotia and *C. minitans* suspension. The number of diseased plants was recorded. According to symptom evaluation scale, ranged from 0 (no disease) up to 10 (100% necrotic leaves or dead plants) the plants were grouped into infection classes, calculating the McKinney index.

In greenhouse trials, “LM 1307” genotype showed less significant susceptibility to Botrytis Gray Mould (0-2% of affected plants), while “Ninja” and “Charmy” showed 4-11% and 16-26% of diseased plants, respectively. The yields were 69.7, 62.7, 55.3 t/Ha, respectively. In laboratory tests, the McKinney index gave the following results: no disease in all untreated plants; 38.3, 54.2 and 89.2% in “LM 1307”, “Ninja” and “Charmy” treated with sclerotia only, respectively; 2.5, 7.5 and 20.8% in “LM 1307”, “Ninja” and “Charmy” treated with sclerotia and *C. minitans*, respectively. In conclusion, the less susceptibility of the genotypes to sclerotial diseases and the use of hyperparasites of sclerotia of phytopathogenic fungi exhibited best results.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is native to the Mediterranean area and was domesticated in Egypt around 4,500 B.C. The first report dates back to the ancient paintings of the Egyptian tombs representing long leaves of lettuce. It is known for certain that a number of different varieties of lettuce were growing by the ancient Greeks and Romans, in 450 B.C. and in the first century A.D., respectively.

Annually, around 1,018,000 ha are cropped under lettuce and harvested in the world (about 50% in China that is at first position), with 210 q/ga in yield (FAO, 2004). In Italy, during 2003, the surface cropped under lettuce was 22,008 ha (over 11% of this area was cultivated under greenhouse) while the harvested production was 4,652,864 q (ISTAT, 2004).

Lettuce is a member of the family *Asteraceae* with five main botanic types: *L. sativa capitata* (L.) Janchen, butterhead lettuce; *L. sativa crispa* L., chrisphead lettuce; *L. sativa longifolia* (Lam.) Janchen, romaine lettuce or cos lettuce; *L. sativa acephala* Dill., leaf lettuce; *L. sativa augustana* All. (= *angustana* Auct.) or *augustana* Reichb. *ex* Nym., known also as Celtic lettuce or asparagus lettuce, celery lettuce, Chinese lettuce, stem lettuce. A large number of lettuce cultivars have been bred and grown all over the world, main for use in salads.

Lettuce for fresh consumption is an important field vegeTable crop in Italy, where it is commonly grown in fields or in greenhouses under irrigated conditions. For field lettuce, the vegeTable crop is seeded in greenhouses and planted out as soon as the bed can be prepared. Some producers seed the lettuce directly into the field. Leaf lettuce is harvested about 50 days after transplanting while head lettuce takes closer to 75 days.

Lettuce diseases studied in the present work were Botrytis Gray Mould and Sclerotinia Drop, two important sclerotial affections of lettuce in greenhouses and in open field. These diseases were controlled by using the genetic improvement for resistance and the biological control. Too often it is assumed that disease management is synonymous with fungicides. Fungicides can provide excellent control of some diseases, but for others they may be ineffective, unavailable or illegal and may induce a gradual increase in the occurrence of fungicide resistance that is recognised when expected levels of disease control are no longer achieved with the recommended dose. Fungicide resistance can arise rapidly and completely so that disease control is totally lost. Sometime fungicide resistance can be a more gradual process resulting in partial loss of control.

Botrytis cinerea Pers. ex Fr. is an important necrotrophic fungal pathogen belonging to the subphylum (imperfect fungi) *Deuteromycotina*, class *Hyphomycetes*, order *Hyphales* (*Moniliales*), family *Moniliaceae*. This conidial anamorphic state or asexual stage of the metagenesis cycle occurs on the lettuce leaves, where erect and cylindrical conidiophores appear. They are 0.5-1.5 mm tall, ending with 2-3 very short branches (each furnished of sterigmas) to which ovoid and hyaline conidia (3-5 per branch, forming a compact glomerulus), 8-15 × 6-10 µm, are attached. The teleomorph or sexual stage of *B. cinerea* is *Botryotinia fuckeliana* (de Bary) Wetz. that belongs to the subphylum *Ascomycotina*, class *Discomycetes*, order *Helotiales*, family *Sclerotiniaceae*, and is characterized by the production of one to several apothecia rising from sclerotium, in spring and early summer. Sclerotia are first whitish, then black, lenticular, 2-7 mm in length and width, 1-3 mm thick. Apothecia are shallow cupulate to plane, pale brown, with disc 1.5-3 mm diameter and stipe 3-15 × 0.5-1 mm. Mature apothecium fruiting surface (hymenium) exposes cylindrical asci 100-150 × 6-10 µm containing unicellular and hyaline ascospores 6-10 × 4-6 µm.

B. cinerea causes significant diseases on a large number of plant species, and it is found and dreaded the world over, especially in high values crops such as lettuce cultivations. *Botrytis* infections impair not only the quantity but more important for the growers today, the quality of vegeTable crops.

Consequently, measure to prevent Gray Mould infections have long since become an essential element in the growing of many horticultural crops. Because of the widespread use of transplants in the early spring, Gray Mould at times can cause significant stand losses in leaf lettuce plantings. This effect is particularly evident with Romaine cultivars as this lettuce type is very susceptible to the disease. The initial symptom of Gray Mould is a water-soaked, brownish-gray rot that occurs on transplant leaves and stems that are damaged during the normal practice of handling and planting. Damaged tissues that are wet or in contact with the soil are especially susceptible. From infected leaves and stems, the pathogen progresses into the healthy crown of the lettuce plant and causes a decay and eventual death of the main stem. A dense, fuzzy gray growth of *B. cinerea* fungus can cover diseased areas. Spores are easily blown by wind, air movement, agitation or water splash.

The conditions of infection also show the great adaptability of the fungus: the conidia germinate at a relative humidity of over 90% and at a temperature of 1 to 30 °C. The optimal temperature appears to be between 18 and 20 °C. This great flexibility is why a certain level of inoculum is present almost everywhere and the epidemiological spread of the disease can only be inhibited when dry conditions prevail. The disease may occur over a wide range of temperatures but it is strongly favoured by fog, light rain or high humidity periods, and moderate temperatures. Therefore, *B. cinerea* is worldwide one of the most important and commonly fungal pathogens found in greenhouses.

B. cinerea can attack lettuce seedlings in seed-bed or in field after the direct seeding. Seedlings can collapse from a soft, tan-to-brown, water soaked rot of the stem at or near the soil line. The typical gray mould soon develops on the decayed tissue. Damping-off of seedlings by *Botrytis* occurs primarily in cold frames where the humidity is high, but can also occur in the field if the seeds are contaminated with sclerotia or mycelium, or if *Botrytis* is present in the soil, living on marcescent vegeTables tissues.

The other important lettuce disease studied in this work is Sclerotinia Drop that is caused by the fungi *Sclerotinia sclerotiorum* (Lib.) De Bary and/or *Sclerotinia minor* Jagger. These fungi can affect lettuce and many other plants, including almost all vegeTables except corn. Sclerotinia Drop is a serious disease, and was first reported in the 1890's in Massachusetts. It is now believed to be found worldwide wherever there is cool, moist weather and lettuce is grown. Infection of the plants occurs mainly as they near maturity, but may occur at any time during the season. Under moist conditions, the entire plant may collapse in two days.

S. sclerotiorum and *Sclerotinia minor* are two important fungal pathogens belonging to subphylum *Ascomycotina*, class *Discomycetes*, order *Helotiales*, family *Sclerotiniaceae*, that are characterized by production of one to several apothecia from sclerotia. The *Sclerotinia* genus apothecia are cup-shaped to funnel-shaped to plane, brownish, stipitate, arising from distinct, free, tuberoid sclerotia with a carbonaceous rind (black) and medulla (white) without remnants of host tissue. The apothecial outer excipulum (ectal excipulum) can be composed by globose to hexagonal cells. Ascospores are hyaline, ellipsoid, unicellular, multiguttulate. Macroconidial anamorphic state is wanting. *S. sclerotiorum* apothecia are solitary or several from each sclerotium with disc 2-10 mm in diameter, plane or recurved and when mature with a central depression; the stipe is 4-30 × 0.5-2 mm. Ectal excipulum is composed of slightly elongated prosenchymateous cells turning out perpendicular to the apothecial surface. The hymenium exposes during the maturation asci 110-150 × 6-10 µm, containing ascospores uniform in size, 9-13.5 × 4-6 µm, binucleate. Apothecia grow in spring or high summer. Sclerotia are tuberoid, very variable in shape and size, 4-25 × 2-10 × 5-15 mm. The fungus can found on a wide range of host plants including over 350 species among 60 plant families. *S. minor* apothecia is one from each sclerotium, with disc 2-9 mm diameter, applanate or recurved and with a central depression when mature, with stipe 1-4 × 1-2 mm. Asci are 125-180 × 7-11 µm with ascospores uniform in size, 8-17 × 5-7 µm, tetranucleate. Apothecia grow in high summer. Sclerotia are tuberoid or irregularly shaped, 0.5-2 mm diameter. The specie is not known from the Nordic countries.

Sclerotinia Drop occurs in all lettuce-production areas of the world when cool moist conditions exist. The fungi can persist in soil for long periods of time in the form of over seasoning sclerotia. *S. sclerotiorum* lesser extent in Central and Southern Italy and *S. minor* can cause disease in a wide variety of different plants, including many vegeTable crop plants.

The name “Sclerotinia Drop” best describes the prominent and most obvious symptom of this disease. The first symptom that is noticed is wilting of the outermost leaves. Before leaves wilt, however, a water soaked area caused by the pathogen fungus as it begins to grow, appears on the stem near the soil or upper root. The fungus will grow from this point down into the roots, and up through the rest of the stem. As the fungus grows into each leaf, rots appear on the base of the each leaf. Subsequently, the leaves begin to droop and wither, and their tips to touch the soil. As the fungus grows up the plant, each leaf is affected in turn. The inner leaves usually remain moist enough for the fungus to completely invade them, and reduce them to a slimy mass. The symptomatology is likely to be observed as the plants gain enough size to cover the majority of the plant bed. Dense masses of white fungal growth appear on the surface of rotted tissue near the soil surface. Hard black irregularly shaped sclerotia are produced on and in the rotted host tissue. The pathogens can be rapidly identified by the size of sclerotia produced on decayed lettuce tissue. *S. minor* produces small sclerotia, whereas the larger sclerotia are produced by *S. sclerotiorum*.

These pathogens carry over from season to season as active mycelium in dead plant tissue and as sclerotia in the soil. Mycelium emerging from germinating sclerotia of *S. minor* and *S. sclerotiorum* can infect lettuce plants directly through senescent lower leaves and through root tissue near the soil surface. Sclerotia of *S. sclerotiorum* can also germinate in shaded areas on the soil surface during wet weather by producing apothecia. Apothecia forcibly release ascospores into the air for a period of 2 to 3 weeks. These ascospores are carried by air currents and deposited on healthy lettuce plants, which subsequently become infected. Sclerotia germinate in moist soil during cool weather. *Sclerotinia* can cause infection from 1 to 30°C, with an optimum temperature range between 15 and 21°C. The optimum pH for sclerotia germination is 6.5. One sclerotium per 100 g of soil is sufficient to cause severe disease, and may survive up to 10 years in dry soil, whereas it decay in moist soil and may rot in less than a year. If the soil remains damp for several weeks, if there are excess weeds increasing humidity around the crop, or if there is a pattern of drying and rewetting, those sclerotia of *S. sclerotiorum* found in the top 5 cm of soil will be stimulated to form apothecia. The apothecia expel ascospores into the air and air currents can carry them to a healthy host where infection may occur. The fungus will attack living tissue, but often infection is initiated via a senescent flower or leaf. *S. minor* does not form apothecia or form one apothecium per sclerotium, and therefore frequently can only infect tissue in direct contact with the soil containing sclerotia.

Regarding the management and the control of Gray Mould and Sclerotinia Drop in lettuce, there are several management strategies that can be implemented to minimize losses due to both diseases. The following are some important indications: 1) accurate sanitation after each lettuce growing cycle by removing any plant debris and keeping the greenhouse clean all year round; 2) provide adequate ventilation spacing the plants, and keep air humidity down avoiding excessive watering and sprinkler irrigation systems which tend to wet the top of lettuce beds. This is especially important when lettuce plants begin to cover a majority of the bed surface. Remember that *B. cinerea* needs 93% RH to germinate and infect plant tissue and wet soil stimulates *S. sclerotiorum* and *S. minor* sclerotial germination and plant infection (optimum range of RH is 95-100%); 3) avoid nitrogen feedings; 4) deep plowing tends to bury sclerotia, which promotes rotting of these fungal propagules and reduces their ability to germinate and cause infection; 5) remove and burn any infected plant from the lettuce field; 6) weed the fields and apply crop rotations with resistant crops such as corn and grasses; 7) chemical (Aloj *et al.*, 2000; Minuto *et al.*, 2000) and physical (Peruzzi, 2000) management tools can provide effective disease control when applied promptly after thinning according to label recommendations; 8) use of resistant varieties, or apply biological control (Budge and Whipps, 2001).

Following the last recommendation about the use of resistant varieties, the lettuce genotypes showing in preliminary trials the best behaviour towards Gray Mould were compared.

Regarding the biological control, we evaluated the effectiveness of the use of *Coniothyrium minitans* Campbell against *B. cinerea* causing Gray Mould and *S. sclerotiorum* and *S. minor* causing Sclerotinia Drop.

C. minitans is a fungus belonging to the subphylum *Deuteromycotina*, class *Coelomycetes*, ordo *Sphaeropsidales*, family *Spaeropsidaceae*, developing a dense mycelium with numerous pycnidia in chains or scattered among the hyphae. Pycnidia are brown to black, becoming carbonaceous with age, immersed to superficial, subglobose to globose, ostiolate, 150-600 µm diameter, when mature containing conidia dark brown in mass. Each conidium is ovoid to ellipsoid, or shortly cylindrical, or nearly globose, smooth to roughened, 4-7 × 2.5-3.5 µm. The conidiogenous are short and composed of cells hyaline, simple, ovoid, ampulliform, enteroblastic, phialidic and arising from the innermost layer of cells lining the cavity of the pycnidium. Pycnidial wall is thick, composed of pseudoparenchymatic tissue with several layers of cells, the outer layers thick-walled and heavily pigmented, the inner layers hyaline and thin-walled. Mycelium, pycnidia, conidiogena and conidia belong to the asexual stage of the metagenesis cycle of the fungus, because the teleomorph of *C. minitans* is not known. The *C. minitans* is a world-wide autochthonous soil microrganism characterized by its pathogenicity towards fungal species producing sclerotia. Production of sclerotia by plant pathogenic fungi constitutes a means of survival in the soil in the period between cropping and the planting of new susceptible crop plants. Hyphal tips of *C. minitans* penetrate the cell walls without the formation of specialized structures causing the protoplasm of the host fungus to disintegrate and the walls to collapse. Infected sclerotia become soft, disintegrate and fail to germinate. Sporulation of the mycoparasite occurs on the surface of and inside the sclerotium, thus providing the inoculum required (spread by water or soil mesofauna) for further infections of sclerotia in the soil. Conidia of *C. minitans* can persist ungerminated in disintegrated sclerotia in a range of soil types for at least a year, and the fungus can be recovered from soil without sclerotia for up to 18 months. It can be assumed that long term survival is largely dependent on the presence of sclerotia of susceptible fungi. *C. minitans* is a highly specialised hyperparasite being its host range restricted to certain sclerotia-forming fungi. *C. minitans* does not produce secondary metabolites with undesirable properties, including toxins. The development of resistance appears unlikely and there is no report from any area of the world describing resistance to *C. minitans*. Never it has been established that *C. minitans* is capable to infect green plants and its isolation from humans or animals has not been reported.

The current paper reports the results of a lettuce greenhouse trial which examined the behaviour of some lettuce varieties towards naturally infections caused by Gray Mould and a laboratory experiment which examined the effect of applications of conidia suspensions of *C. minitans* for controlling Sclerotinia Drop on plants grown in soil artificially inoculated with sclerotia of *B. cinerea*, S. *sclerotiorum* and *S. minor*.

MATERIALS AND METHODS

Research was carried out to evaluate the behaviour of some varieties and hybrids of lettuce against Gray Mould, in greenhouse. The compared lettuce genotypes derived from a massal selection (carried out during the former years) of phenotypes showing behaviours of tolerance or less susceptibility towards Botrytis Gray Mould (Fiume *et al.*, 1999). Three genotypes of lettuce were compared, replicated four times in a randomized complete block experiment design, in order to search genotypes showing the best behaviour to escape the disease. The compared genotypes were: "Charmy", "LM 1307" and "Ninja". Each plot was 8.98 m^2 (3.12 x 2.88 m), included 156 plants (0.24 m between rows and 0.24 m on the row), equal to 13 rows and 12 plants per row, with density of 173,719 plants per hectare. Transplant of lettuce plantlets with 3-5 leaves was carried out at end of January on black plastic mulch. Irrigation and fertilization (by minimum dose of highly soluble mineral fertilizers) were carried out by using plastic holed hoses placed under the plastic mulch, each 2 rows of lettuce plants (spaced out 0.48 m). Phytopathological observations and yield were registered per each plot.

The phytopathological observations started about 30 days after the plantlet transplant (February 27th) and continued with weekly interval up to the harvest. The number of diseased plants was registered for each plot, calculating the percentage of affected plants and building the trend of the gray mould attack per each lettuce genotype. The diagnosis of the disease was carried out by observing the plants that showed typical rot on the leaves where a dense gray mould covering the disease area appeared. The microscopy examination of the mould exhibited typical long and cylindrical conidiophores, ending with 2-3 short branches to which smooth-ovoid-hyaline-unicellular conidia were attached, by sterigmas. The conidiophores and conidia were ascribable, in relation to form and size, to *B. cinerea.*

The harvest was executed for all the plots on April 7th registering the healthy plants, the yield, the average weight of the lettuce head and the quality of the lettuce head. This last parameter was more important than the weight because poor quality cause generally unmarketability while the relative low weight of the lettuce head and their high quality evidence a significant and favourable influence on the marketability. The quality of lettuce head was graded on a 1 to 5 scale, with 1 = poor and 5 = excellent. The quality data were completed counting and weighing (fresh and dry weight) the leaves, splitting the external from the internal leaves of each lettuce head. The external were the leaves that envelop and protect the lettuce head, intense green, poor in water, which are normally eliminated during the food preparation for hygienic reasons, because damaged, less appetizing and palaTable. The internal were the leaves following the external towards the head inside. They are protected from the injuries by the crown of external leaves. They are crisp, pale green to white, rich in water, tasty, appetizing and palatable.

The biological control trial was carried out in laboratory controlled conditions (18-22°C, 85-90% relative humidity and 12 h photoperiod) and started isolating *B. cinerea*, *S. sclerotiorum* and *S. minor* from infected lettuce plants on PDA (potato, dextrose, and agar) medium. Small pieces of tissue were taken from the edge of a lesion of lettuce plants grown under plastic greenhouse to isolate the three fungus pathogens. The three isolates grown onto PDA plates and after incubation for 6 weeks allowed obtaining sclerotia that were used for testing the pathogenic ability of *C. minitans*. This mycoparasite fungus was used by application of Contans®WG that Prophyta Company has developed and produced in Germany as first biological fungicide. Contans®WG is a water-soluble granule with a specific antagonism action against sclerotia of plant pathogens, containing 1×10^9 active spores/g of *C. minitans* dried on glucose. Contans®WG was tested isolating the granule on PDA and OA (oat, agar) plates where the typical mycelia, pycnidia and conidia, ascribable to *C. minitans*, appeared.

Then, 27 small plots 30 cm in diameter each, with disinfected soil, were performed. Only 18 plots were each inoculated with 9 sclerotia coming from dishes Petri in which the pathogen fungi had grown: 3 sclerotia of *B. cinerea*, 3 of *S. sclerotiorum* and 3 of *S. minor*. The sclerotia of the three pathogen fungi are easily distinguishable: those of *B. cinerea* are lenticular and of a size ranged between the sizes of the sclerotia of the other two pathogens; those of *S. sclerotiorum* are tuberoid and much bigger; those of *S. minor* are tuberoid and smaller. The sclerotia were inoculated on the surface of plot soil, and then, only 9 plots of the 18 plots inoculated with sclerotia, were treated by using Contans® WG at 6 kg/ha. Therefore, 0.36 g of Contans® WG were suspended in water up to 4.5 l. The suspension was distributed in equal part among the 9 plots (0.5 l per plot). Each plot received 0.04 g of Contans® WG. On the following day, the inoculated soil of each plot was mixed up to 10 cm in depth, and was kept wet until transplant of the lettuce plantlets.

About 15 days late (June 13th), three lettuce genotypes were transplanted on all the plots, performing a randomized complete block experiment design with 3 replications where for each genotype were compared: 1) untreated plots; 2) treated plots with sclerotia only; 3) treated plots with sclerotia and *C. minitans* suspension. For each plot 4 lettuce plantlets were transplanted.

The number of diseased plants was recorded 40 days after transplant. The severity of Gray Mould and Sclerotinia Drop was estimated for each plant. All the plants were grouped into infection classes, calculating the frequencies according to the following symptom evaluation scale that ranged from 0 to 10: 0 = no disease symptoms; 1-9 = 10-90% necrotic leaves or lesion areas on lettuce plants; 10 = 100% necrotic leaves or dead plants. Severity and diffusion of infection were obtained by McKinney index (I) that was calculated by using the following relation:

$$I = \frac{\Sigma (f \cdot v)}{N \cdot X}$$

where:

f = infection class frequencies;
v = number of each class;
N = amount of observed plants;
X = highest value of the evaluation scale.

Meteorological data were detected in greenhouse during the trial period: minimum and maximum air temperature (°C) and relative humidity (%) were registered by using a hygrothermograph (Siap 7006) placed to 50 cm on the soil surface.

Data were compared using Duncan's test for multiple comparison with MSTAT statistical analysis program. Percentage data were transformed to angular values before of the variance analysis.

RESULTS

The meteorological conditions in plastic greenhouse were favourable for development of lettuce disease (Figure 1).

From February to April (period in which phytopathological observation were carried out) the minimum and maximum temperatures ranged from 5 up to 15°C and from 20 up to 30°C, respectively. The temperature average was 12-24°C. The relative humidity exhibited values between 42 and 68% (minimum), about 100% (maximum), and average 67-84% (Figure 1).

The genotype "LM 1307" showed the best behaviour against gray mould. The number of healthy plants, the yield and the production quality were significantly highest. The varieties that followed were "Ninja" and "Charmy" (Table 1).

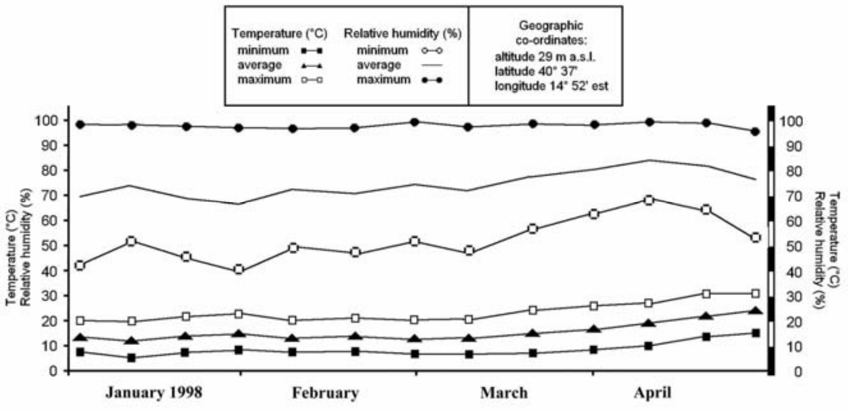

Figure 1. Data of temperature and relative humidity detected during the greenhouse trial.

Table 1. Healthy and markeTable plants, gray-mould diseased and unmarkeTable plants, yield and average weight of a head, head quality evaluation (*).

Lettuce genotypes	Healthy plants (no./Ha)	Diseased plants %	Yield (t/Ha)	Head average weight (g)	Head quality (1)
Charmy	128,412 Bc	26.1 Bc	55.3 Bc	430.6 Aa	3.7 Bc
LM 1307	170,595 Aa	1.8 Aa	69.6 Aa	408.0 Ab	4.9 Aa
Ninja	153,848 Ab	11.4 Ab	62.6 Ab	406.9 Ab	4.4 Ab

(*) Value separation in columns by Duncan's multiple range test (P≤0.01 capital letters; P≤0.05 small letters).
(1) Head quality graded on a 1 to 5 scale with 1 = poor and 5 = excellent.

The average number of leaves and their dry weight, the percentage portioning out between external and internal leaves of the lettuce head are reported in Table 2. The "LM 1307" selection showed the highest number of leaves. Observing the dry weight of the leaves no significant differences were noticed among the genotypes. Referring to the number of leaves of each lettuce head, the "LM 1307" selection showed significantly the lowest percentage of the external leaves and the highest percentage of internal leaves. Referring to the weight of leaves, no significant differences were observed between "LM 1307" and "Ninja", while "Charmy" showed different significantly values, whether external leaf percentage or internal leaf percentage.
The study of the epidemiologic trend of each lettuce genotype is represented in the Figure 2. The "LM 1307" selection was affected by Gray Mould for plant percentage negligible: the number of diseased plants was around 1%, ranging form 0 to 2%. The "Ninja" variety showed values of diseased plants ranging from 4 to 11%; the curve increased up to 35 days after the transplant when showed a point of inflection that was repeated 53 days after transplant. The "Charmy" variety showed, during the observation period, a percentage of plants affected by *B. cinerea* that ranged from 16 to 26%. Two point of inflection were observed, 35 and 46 days after plantlets transplant.

The infection trend between the two points of inflection was constant showing about 20% of infected plants.

Table 2. For each head of the compared genotype: average number of all the leaves, dry weight average of all the leaves, leaf number splitted between external and internal (in percentage), leaf weight shared out between external and internal (in percentage) of the lettuce head (*).

Lettuce genotypes	Leaf average number	Leaf dry weight (g)	Leaf number %		Leaf weight %	
			External	Internal	External	Internal
Charmy	39.3 Ab	3.4 Aa	36.4 Aa	63.6 Bb	35.5 Ab	64.5 Aa
LM 1307	41.3 Aa	3.7 Aa	33.9 Bb	66.1 Aa	36.9 Aa	63.1 Ab
Ninja	40.6 Aab	3.5 Aa	35.2 Aa	64.8 Bb	36.7 Aa	63.3 Ab

(*) Value separation in columns by Duncan's multiple range test (P≤0.01 capital letters; P≤0.05 small letters).

The healthy plants ranged up to 33.3% and the McKinney index ranged from 38.3% ("LM 1307" selection) to 89.2% ("Charmy" variety) for plots treated only with sclerotia of the three pathogens. The plots that were undertaken to biological control with *C. minitans* showed the highest percentage of healthy plants and lowest McKinney index that ranged from 66.7% "Charmy" cultivar) to 91.7% ("LM 1307" selection) and from 2.5 ("LM 1307" selection) to 20.8% (Charmy genotype), respectively. No diseased plant appeared for untreated plots.

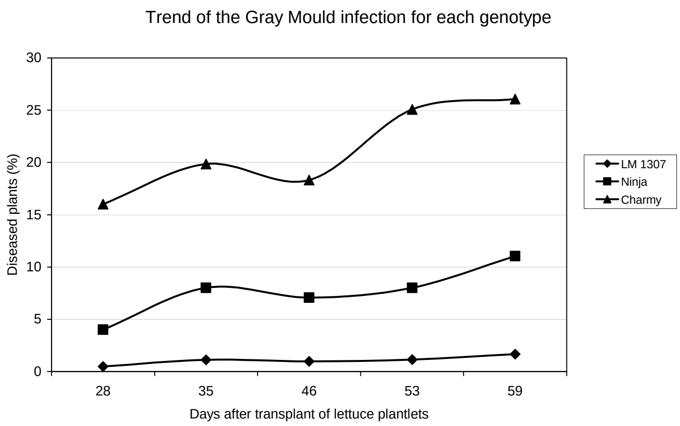

Figure 2. Trends of the gray mould infection caused by *B. cinerea*, one for each of the three compared genotypes of lettuce.

Statistical differences were highly significant (P≤0.01) when were compared the data of the soil with sclerotia only and the data of the soil with sclerotia and Contans, for healthy plants and McKinney index, respectively, and for each genotype (Table 3).

Table 3. Effect of Contans® WG on control of Gray Mould and Sclerotinia Drop.

Lettuce genotypes	Untreated	Soil with sclerotia only		Soil with sclerotia and Contans	
	diseased plants (%)	healthy plants (%)	McKinney index (%)	healthy plants (%)	McKinney index (%)
Charmy	0.0	0.0	89.2	66.7	20.8
LM 1307	0.0	33.3	38.3	91.7	2.5
Ninja	0.0	16.7	54.2	83.3	7.5

DISCUSSION

The environmental conditions of the plastic greenhouse showed favourable values in relation to development of Gray Mould on lettuce. The relative isolation of the greenhouse inside in order to retain the heat during the winter and to protect the plants against infestations (aphids), the consequent absence of air movements and the irrigation practice increased the maximum values of the relative humidity up to 100% as well as the temperature that showed values favourable to the germination of the conidia. Favourable humidity and temperature was the major responsible of the Gray Mould diffusion on lettuce in greenhouse. The high density of the plants (173,719 plants/ha) contributed to increase the relative humidity values too.

The genotype "LM 1307" showed the lowest number of diseased plants and the best production quality. It exhibited tolerance and less susceptibility to gray mould. The tolerance allowed assigning the highest score for quality. The head weight was not higher than other genotypes but this no cause markeTable problems (Table 1). Head lettuce is usually marketed as number of heads per box. The weight of lettuce head is not as critical as quality. The selection "LM 1307" showed a good behaviour throughout the trials. Even if the head weight was not highest, the quality was unaffected resulting good. The evaluation of good quality was confirmed by examination of the Table 2. "LM 1307" showed the highest number of all leaves of the head and a dry weight no significantly different than other genotypes. Besides, the number of internal leaves was highest while their weight was lowest. Consequently, the eaTable leaves of "LM 1307" were richer in water and crisper, tasty, appetizing, palaTable than other genotypes. Referring to the weight of leaves, the lowest value of the external leaf percentage and the highest value of the internal leaf percentage, compared with the leaf number, indicated the lowest quality of "Charmy".

The behaviour of the compared genotypes is distinctly clear showing the absolute susceptibility towards Gray Mould for "Charmy" and a good tolerance for the lettuce selection named "LM 1307". Intermediate characteristics were noticed for "Ninja" (Figure 2).

In laboratory trial, the biological control against Gray Mould and Sclerotinia Drop by using *C. minitans* showed a good effectiveness. The percentage of healthy plants was significantly highest and the McKinney index was lowest for plots treated with Contans® WG. The best results were obtained for the lettuce "LM 1307". Regarding the McKinney index, "LM 1307" showed a favourable interaction between genotype and biological control. The response for applying the two treatments (genotype with tolerance to Gray Mould and biological control with *C. minitans*) was not the simple sum of the responses to each treatment (Table 3).

In conclusion, resistant cultivars and biological control are the most desirable ways to control plant diseases, with the advantage of a wider public acceptance and reduced environmental contamination. "LM 1307" selection did not evidence high resistance. It showed a good behaviour with a lesser susceptibility compared to other genotypes. Therefore, the global result was positive in relation to the better quality of the yield that represents a more relevant factor respect to the quantity of the yield. The less susceptible genotype and the biological control together can significantly improve the yield quality and the environmental conditions. The effective management strategies for Gray Mould and Sclerotinia Drop should include the planting of less susceptible cultivars that reduce lesion areas on the plants and the biological control that increases the biodiversity and reduces the epidemic diffusion.

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