농약과학회지 (Korean J. Pestic. Sci.)
Vol. 21, No. 2, pp. 224-231 (2017)
Open Access https://doi.org/10.7585/kjps.2017.21.2.224
ORIGINAL ARTICLES

Online ISSN 2287-2051
Print ISSN 1226-6183

Exported Strawberry Gray Mold Decay Related Spore Density and Disease Incidence in Cultivation Field

Chang-Wook Jeon[1], Da-ran Kim[2], Jung Jun Park[2], Nam Jun Kang[3] and Youn-Sig Kwak[1,2]*

[1]Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Korea
[2]Department of Plant Medicine and Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Korea
[3]*Department of Agricultural Plant Science and Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Korea*

(Received on May 22, 2017. Revised on June 27, 2017. Accepted on June 29, 2017)

Abstract Exports of strawberries are currently expanding and have risen by 65% in the last few years. However, the occurrence of gray mold disease in packed strawberries has emerged. Gray mold disease, caused by *Botrytis cinerea,* leads to the decomposition of strawberries, reducing the total harvesting of the fruit by up to 50%. In this study, to determine the correlation between cultivation fields and a packing center of disease incidence and spore density, investigation were conducted for two consecutive years. The strawberry cultivation fields showed the highest disease incidence from March to May in both years. However, in both fields the pathogen spores in the air showed similar density during cultivation periods as 10^5 cfu/L of air in the first year and 10^4 cfu/L of air for the second year. In the packing center, the spore density patterns were similar to those in the fields. Temperature and humidity emerged as having the most correlation with incidence of the disease in the fields. The major finding in this study was that the source inocula of packed strawberry were derived from the cultivation fields. The findings can be utilized to effectively control gray mold decay in export strawberry production.

Key words export strawberry, gray mold disease, postharvest, spore density

<< ORCID
Youn-Sig Kwak
http://orcid.org/0000-0003-2139-1808

Introduction

A significant proportion of South Korea's strawberry (*Fragaria x ananassa*) industry involved cultivation of this fruit on open fields until 1985. From the 1990s onwards, however, the use of vinyl greenhouses and machinery became the norm, and the production of strawberries improved, leading to higher profits. Strawberries have become much more consumed globally in the past two decades and an increasingly internationally traded commodity. Diseases that commonly infect strawberries for export are inflicting considerable damage on the domestic economy (Sistrunk and Morris, 1985). The incidence of gray mold at strawberry plantations is 10-15% on average, and the percentage affected is continuing to rise (Kim et al., 2015). For this reason, a variety of treatments before and after harvest have been sought to maintain the freshness of strawberries. For example, the low-temperature storage method which is basically a precooling strategy implemented immediately after harvesting has been used, but to not much effect (Kedar, 1992).

B. cinerea is the fungus that causes gray mold decay, and there is a wide range of host species in vegetables such as cucumbers, peppers, tomatoes, and various crops such as grapes, blueberries, and so on. *B. cinerea* has been reported in 86 species of economic crops such as red pepper, strawberry, apple, cucumber, tomato and ginseng (The Korean Society of Plant Pathology, 2009). Gray mold is a major disease of crops

*Corresponding author
E-mail: kwak@gnu.ac.kr

occurring in various parts of infected plants such as leaves, stems, and fruits (Coley-Smith et al., 1980; Elad et al., 2004; Guillem et al., 2007). The pathogen is also the cause of storage decay that decays the fruits and vegetables that are being stored after harvesting, resulting in very large economic losses (Jarvis, 1977). For this reason, the global market of disinfectants for controlling or eradicating gray mold is now worth US$1.5-2.5 billion and recently, research and development to control this disease has increased (Nam et al., 2011).

The gray mold pathogen spreads through the conidia as primary source in the strawberry cultivation and brown spots are formed on the fruit and the calyx (Braun and Sutton, 1987). While studies on the control of gray mold in cultivated strawberries have been done, but no report has been published on the correlation between disease incidence and pathogen spore density, for strawberry cultivation and packing areas. In this study, *B. cinerea* spore density and the incidence of disease during cultivation and in packing areas were investigated for 2 years to track the main source of infection that is affecting strawberry exports.

Materials and Methods

Measuring the pathogen spore density in the air of strawberry greenhouses

Two greenhouses for strawberry cultivation and a packing area was located in Jinju, South Korea. These greenhouses were 10 × 88 meters and had 6 high-bed cultivation lines system. Tests were carried out in three lines and spores were collected from each line at three locations, one at the exit, one at the center and one at the farthest point from the exit. Also, in order to investigate the differences between spores' density according to the micro-meteorological conditions, experiments were carried out in two greenhouses, named greenhouse A (GA) and greenhouse B (GB). GA and GB planted cultivar Maehyang with a high-bed cultivation system and the general management for cultivation was the same, except GA had installed customized machinery to control temperature and humidity. The export strawberry packing facility was located in the same area as the greenhouses. The packing facility was 30 × 40 m in size and had 6 screening and packing lanes.

Collection of spores and survey of the gray mold disease incidence were conducted from November 2014 to May 2015 and from November 2015 to May 2016 every two weeks. Three air spore samplers (UCK Bio-Culture^{TM}UPump, USA) were used to place in the strawberry greenhouses or the packing facility. *B. cinerea* spore selective media, Botrytis Spore Trap Medium (BSTM; glucose 2 g, $NaNO_3$ 0.1 g, K_2HPO_2 0.1 g, $MgSO_47H_2O$ 0.2 g, chlorampenicol 0.2 g, pentachloronotrobenzene 0.02 g, Maneb 80 0.02 g, Rubigan 0.1 ml, tannic acid 5 g, agar 20 g per 1 L) (Kim et al., 2015), inside the sampler. Then the sampler was activated to suck air for 2 min. The media's collection of airborne spores was done in triplicate. When this finished the inhaled air, the BSTM plates were sealed with parafilm and incubated at 28°C for 7 days, and the number of colonies on the fungus was measured and the value was calculated in Colony Forming Units (CFU Log / L of air).

Fig. 1. Gray mold disease symptoms of strawberries. (A: Crown, B: Fruit, C: Primary inoculum on strawberry fruit in packing place)

Investigating the incidence of gray mold disease

The strawberry greenhouse and the survey period were the same as described above and a study on disease incidence was conducted in both GA and GB (Fig. 1). Disease incidence was investigated by randomly selecting 100 strawberries from each greenhouse at the investigation date every year and the experiment was conducted in triplicate (Kim et al., 2015).

Strawberries that were packed at the distribution center in Sugok-myeon, Jinju-si, Gyeongsangnam-do were brought into the laboratory and stored at low temperature (4°C) and room temperature (21°C) for 10 days, respectively. After 10 days of storage, the number of strawberries in each package was counted. Then, the number of strawberries with gray mold was counted and the number of diseased fruits in the total number of fruits was converted into percentage. The treats at low temperature (4°C) and at room temperature (21°C) were packed with 12 fruits respectively and they were examined by a total of 8 repeats.

Micro-meteorological analysis of disease incidence and spore scattering by multiple regression analysis

Multiple regression analysis was performed using forward and stepwise methods and the differences in the environmental factors leading to gray mold disease and spore scattering in the

strawberry cultivation area were analyzed using total of 10 environmental factors [soil surface-humidity, electrical conductivity (EC) and temperature, inside the soil-humidity, EC and temperature, atmosphere-temperature, humidity, solar and CO_2 concentration]. In addition, the differences in environmental factors' impact on spore scattering in the packing area were investigated using three environmental factors-temperature, humidity and CO_2. All environmental factors were automatically measured every 5 min by customized sensors (Elsys Co., Korea). Based on this, the micro-meteorological factors significantly influencing the incidence of gray mold disease and spore scattering were analyzed through principal component analysis.

Statistical Analysis

Statistical analysis was done using ANOVA analysis (Tukey's HSD) according to the complete randomization method for each treatment and graphs were generated using the Sigma Plot (Systat Software, Inc., Chicago, IL, USA) program.

Results

Investigation of density of *B. cinerea* spores in strawberry plantations for export

The results of the first-year experiment showed that the density of *B. cinerea* spores in GA ranged from 10^5 to 10^6 (Log CFU/L) from December 3, 2014 to January 10, 2015 and it was investigated from 10^4 to 10^6 on the next date of collection. The density result of *B. cinerea* spores in GB showed that spore density was 10^5 to 10^6 from December 3, 2014 to January 15, 2014 and it was investigated from 10^4 to 10^5 on the next date of collection. While the spore scattering period in both greenhouses lasted from December to January, the increase in gray mold began in late February. The reason for this was due to time differences. The results of the second year experiment showed that the *B. cinerea* spore density of GA ranged from 10^2 to 10^3 from November 4, 2015 to February 11, 2016 and it was investigated from 10^4 to 10^6 from March 9, 2015 to May 6, 2016. The spore density of GB ranged from 10^2 to 10^3 from November 4, 2015 to February

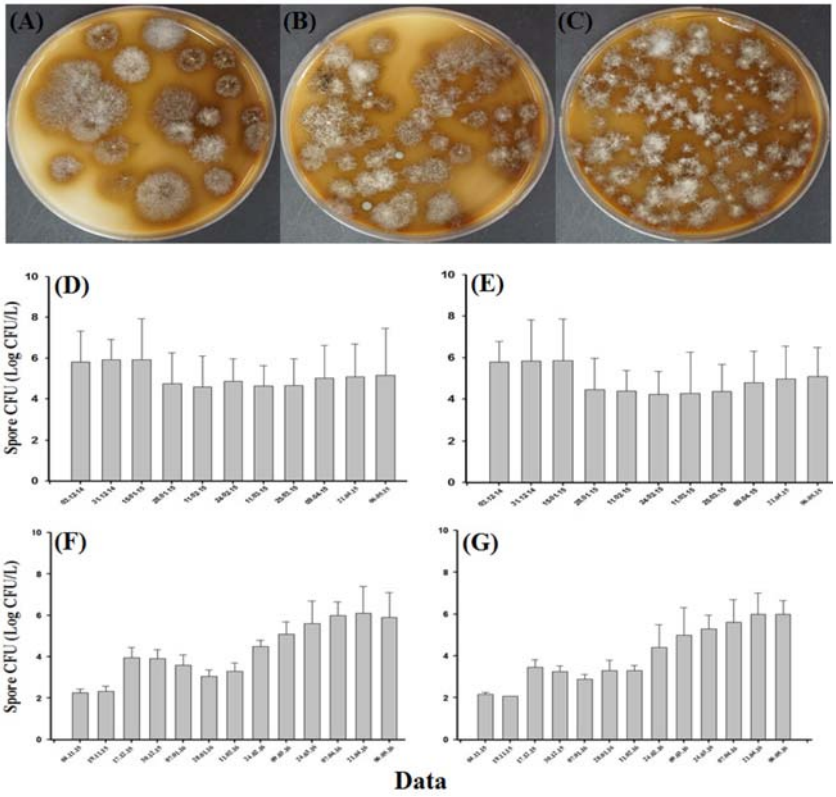

Fig. 2. Floating spore densities of gray mold pathogen in strawberry cultivation fields on BSTM (A,B,C) media. BSTM media were incubated at 28°C for 7 days. Average floating spore densities during survey periods (D, E, F, G). D: 2014~2015 Greenhouse A (no dehumidifying heater installed), E: 2014~2015 Greenhouse B (dehumidifying heater installed), F: 2015~2016 Greenhouse A (no dehumidifying heater installed), G: 2015~2016 Greenhouse B (dehumidifying heater installed). Statistical analysis With Tukey's HSD (p=0.05), vertical bar indicates relative standard errors.

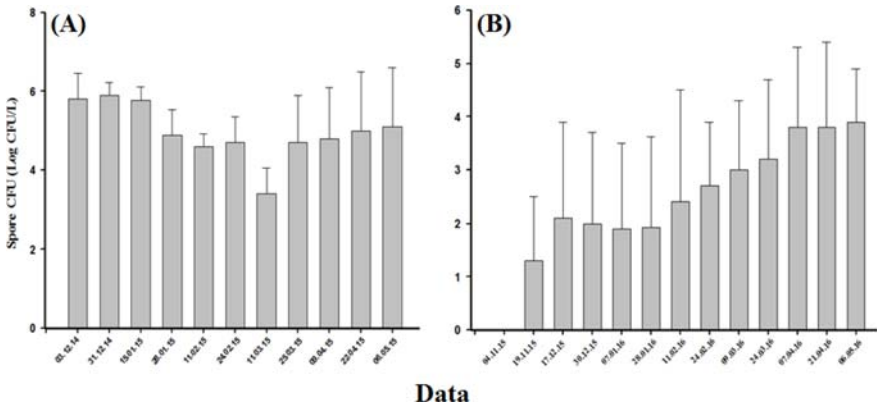

Fig. 3. Floating spore densities of gray mold pathogen in sorting and packing workplace on BSTM media. A: 2014~15 sorting and packing workplace, B: 2015~2016 at packing place. Statistical analysis With Tukey's HSD (p=0.05), vertical bar indicates relative standard errors.

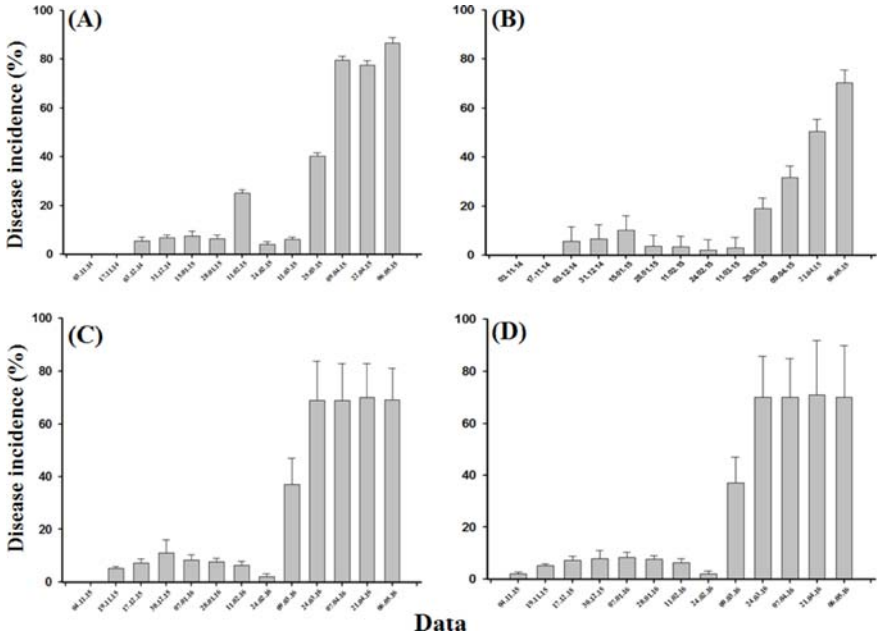

Fig. 4. Gray mold disease incidence on strawberries. A: 2014~2015 Greenhouse A (no dehumidifying heater installed), B :2014~2015 Greenhouse B (dehumidifying heater installed), C: 2015~2016 Greenhouse A (no dehumidifying heater installed), D: 2015~2016 Greenhouse B (dehumidifying heater installed). Statistical analysis with Tukey's HSD (p=0.05), vertical bar indicates relative standard errors.

11, 2016 and it was investigated from 10^4 to 10^6 from March 9, 2015 to May 6, 2016. (Fig. 2). Spore scatterings in both greenhouses had increased since February 2016 and the incidence of gray mold disease rapidly increased from early March in both greenhouses.

Investigation of density of gray mold disease in packing place

The first-year experiment showed that the density of *B. cinerea* spores in the sorting and packing place was investigated from 10^5 to 10^6 in spore density from December 3, 2014 to January 15, 2015 and it was investigated from 10^4 to 10^5 from January 28, 2015 to February 24, 2015. Then, from March 25 to May 6, it was investigated from 10^4~10^5. The results of the second-year experiment showed that the spore density in the packing place ranged from 10^1 to 10^2 from November 4, 2015 to February 24, 2016 and it was investigated from 10^3 to 10^4 from March 9, 2016 to May 6, 2016 (Fig. 3).

Investigation of incidence of gray mold disease in strawberry cultivation fields

The results of the first-year experiment showed that the incidence of gray mold disease in GA ranged from 6% to 7% between December 3, 2014 and January 28, 2015. The

incidence of disease rose to 25% per the result on February 11, 2015 but then fell again until late March. Since March 25, 2015, the rate of disease incidence increased sharply to 86%. It emerged that GB recorded disease incidence of less than 10% from December 3, 2014 to March 11, 2015, but it increased from March 25, 2015 and reached 70%. GB had 16% less disease than GA. In the second year of the experiment, gray mold disease in GA was 0 to 14% from November 4, 2015 to February 24, 2016 but results for March 9, 2016, reported that the incidence of disease increased sharply to 38%, finally reaching approximately 70%. The investigation's result for the incidence of gray mold disease in GB reported 0 to 11% from November 4, 2015 to February 24, 2016, while the disease rapidly increased to 37% on March 9, 2016. Then the incidence of the disease increased by approximately 70%. The incidence of gray mold disease began to increase rapidly from early March 2016 in both greenhouses. Although from early February to early March in 2016, the control of gray mold was actively carried out using a chemical strategy, gray mold disease continued to increase from then on (Fig. 4).

Investigation of disease incidence depending on the storage temperature of packed strawberries in sorting and packing workplaces

Referring to the first-year experiment's results, the storage temperature experiment was conducted at low temperature and room temperature for packaged strawberries in the laboratory. The percentage of disease incidence of fruit stored at room temperature was 69-100%, and 0-17% at low temperature (Fig. 5A). The result of the second-year experiment indicated that the disease occurred 100% at room temperature after January 2016 and the incidence of disease was less than 10% at low temperature (Fig. 5B). Thus, the disease incidence rate of gray mold was remarkably small at low temperature storage but higher at room temperature storage.

Multiple regression analysis of spore scattering and disease incidence

In GA, temperature and humidity were identified as independent variables that have a significant influence on the amount of *B. cinerea* spores. Furthermore, the independent variables affecting disease incidence also appear to be humidity and temperature (Table 1). In GB, temperature and humidity were identified as being independent variables that wield a significant influence on the amount of *B. cinerea* spores. Here the independent variables affecting disease incidence also appear to be humidity and temperature (Table 2). Then, since the multiple regression analysis showed that the significance is below 0.05, this result was considered to be important. Relative humidity in the packing facility was the

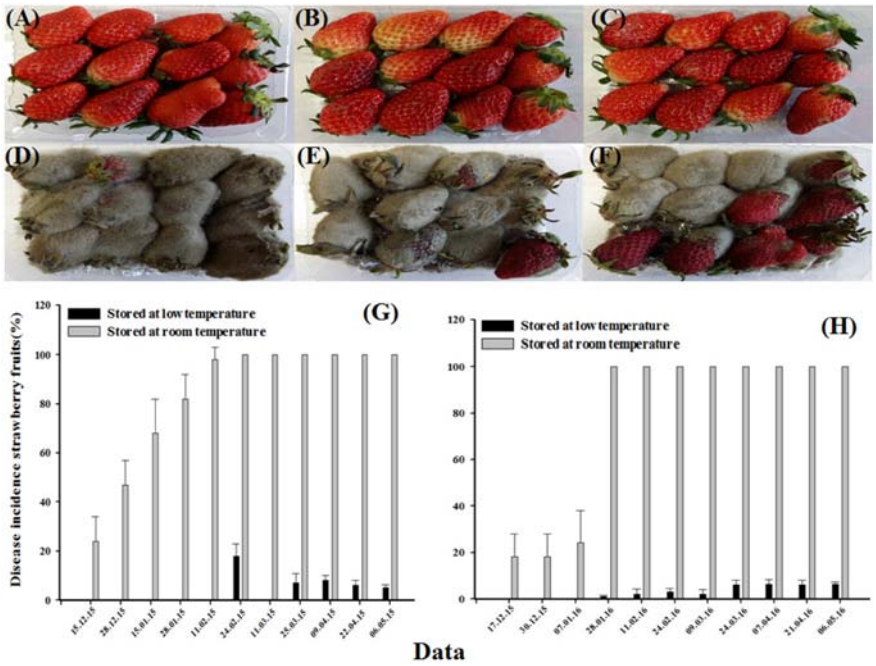

Fig. 5. Gray mold disease incidence based on storage temperature conditions. Packed strawberries were stored at low temperature (4°C) for 10 days (A, B, C). The packages were stored at room temperature for 10 days (D, E, F). Decay ratio of gray mold disease in stored strawberries for both temperature conditions (G: 2014~15, H: 2015~16).

Table 1. Multiple environmental factors analysis in strawberry greenhouse A*

Summary of forward selection											
Dependent variable: Spore Number						Dependent variable: Disease incidence					
Step	Variable entered	Number vars	C (p)	F value	Pr > F	Step	Variable entered	Number vars	C (p)	F value	Pr > F
1	STD[a]	1	20.5373	8.05	0.0177	1	STD[a]	1	26.0676	11.17	0.0075
2	SM[b]	2	10.9406	6.16	0.0349	2	ACO_2[d]	2	14.4477	5.99	0.0369
3	SEC[c]	3	5.4461	6.35	0.0358	3	SM[b]	3	3.9390	12.60	0.0075

*: Among 10 environmental factors, only statistically significant factors are presented.
[a)]SMD for inside soil moist; [b)]SECD for inside soil EC; [c)]SE for soil surface EC; [d)]ST for soil surface temperature.

Table 2. Multiple environmental factors analysis in strawberry greenhouse B*

Summary of Forward selection											
Dependent variable: Spore Number						Dependent variable: Disease incidence					
Step	Variable entered	Number vars	C(p)	F value	Pr > F	Step	Variable entered	Number Vars	C(p)	F value	Pr > F
1	SMD[a]	1	18.5135	16.27	0.002	1	ST[d]	1	62.6100	10.74	0.0074
2	SECD[b]	2	5.5946	11.58	0.006	2	SECD[b]	2	37.4127	6.12	0.0328
3	SM[c]	3	3.8657	4.02	0.08	3	SM[c]	3	17.6888	4.74	0.0611

*: Among 10 environmental factors, only statistically significant factors are presented
[a)]SMD for inside soil moist; [b)]SECD for inside soil EC; [c)]SE for soil surface EC; [d)]ST for soil surface temperature.

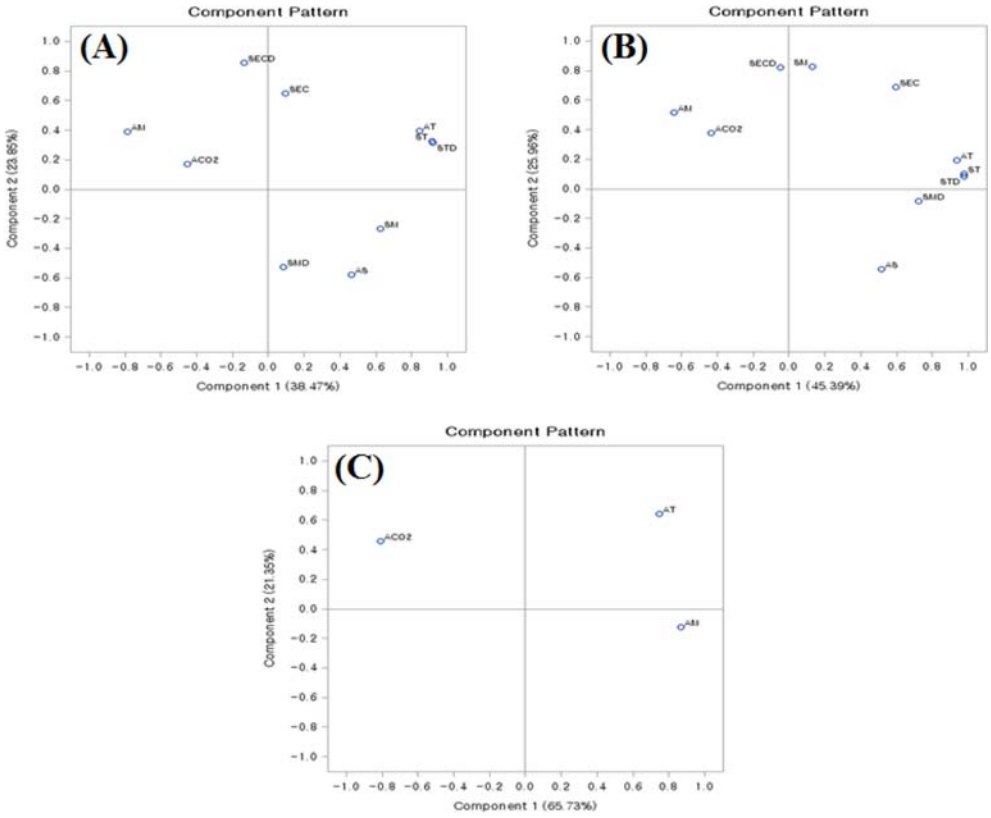

Fig. 6. Principle analysis between diseases and environmental factors in strawberry cultivation. A: Greenhouse A (no dehumidifying heater installed), B: Greenhouse B (dehumidifying heater installed), C: Sorting and packing workplace.

most affecting environmental factor in the spore disperse. However, the result was not consistent with statistical analysis in both years.

Principle analysis of the incidence of gray mold disease in strawberry plantation and micro-meteorological environment from spores produced

While the two greenhouses had different moisture conditions, temperature and relative humidity showed a similar correlation with spore scattering and disease incidence. If dehumidification is not available, the correlation between quantity of light and CO_2 will be reversed, but it was confirmed that no complete correlation was established when dehumidification was not available. If the micro-meteorological environment was not well controlled by a dehumidifier, only temperature was grouped into the same location among nine independent and it was confirmed that the incidence of gray mold and spore scattering were affected by temperature in GA. If the micro-meteorological environment was well controlled by a dehumidifier, only temperature was grouped into the same location among nine independent variables and it was confirmed that the incidence of gray mold and spore scattering was affected by temperature in GB. It was confirmed that interference by temperature occurred in both greenhouses (Fig. 6). In the case of the packing workplace, there was some overlap between temperature and humidity. Overall, the fact that temperature and humidity were highly involved in the microenvironment and greatly influenced the incidence of gray mold disease and spore scattering was confirmed by the PCA results where multiple regression analysis and micro-meteorological data were used to evaluate the impact of humidity.

Discussion

Strawberries are one of the most profitable cash crops grown in agriculture. The crop has small sized fruits and much economic value but is subject to many fungal diseases, such as anthracnose, gray mold, powdery mildew and Fusarium wilt, etc. Gray mold disease is caused by *B. cinerea*, which is an airborne fungal pathogen. The pathogen conidia are horizontally and vertically distributed in the atmosphere or by spores and mycelia are dispersed by insects (Burt et al., 1997). In one previous study, *B. cinerea* spores were affected by environmental conditions and the spores were trapped to monitor their behavior in fall and winter (Kerssies, 1993). Greenhouses use many chemical control methods to eradicate or minimize gray mold disease, for example acibenzolar (Muñoz and Moret, 2010), anilinopyrimidines pyrimethanil and cyprodinil (Tripathi and Dubey, 2004). There are many restraints imposed on export systems because pesticide residues must be strictly controlled in importing countries. At the same time, postharvest disease does appear in 50% of stored strawberries (Hwang and Ku, 2004). In a recent study, biological control and integrated pest management systems have been highlighted in the gray mold disease (El-ghanam et al., 2015). In this study, we confirmed that some environmental factors are critically involved in controlling diseases and pathogen conidia density.

At first, gray mold disease incidence and spore density of atmosphere were monitored using environmental factors during the cultivation periods in two greenhouses. Temperature and moisture were the two variables that influenced the results the most. Incidence of disease had two major requirements for the germination of fungus spores - temperature and humidity (Ayerst, 1969; Hirst, 1953; Pasanen et al., 1991). Our results indicated that the inoculum density persisted but disease incidence increased from May to March in both years, thus suggesting that continuous observation and regulation of temperature and humidity may be the key to reducing disease occurrence. Secondly, the packing place was recognized as requiring proper management to reduce fungi contamination of the strawberries (Kim et al., 2015). Many studies examining chemical control and irradiation methods have been published, such as those using chlorine dioxide (ClO_2), sodium carbonate, sodium bicarbonate, active chlorine and sorbic acid (Rosslenbroich and Stueble, 2000). But our results suggested that irradiation of *B. cinerea* inoculum in the packing place is less important. Because, conidia density of *B. cinerea* were remained relatively constant during survey periods in the packing place. However, all the packaged and stored strawberries incurred symptoms of gray mold disease at room temperature and were not affected by environmental factors. It was also found that when the density of the gray mold disease increased in the strawberry plantation, the rate of decay of packed strawberries also rose. This suggests that active control measures should be implemented on strawberry plantations rather than at the packing place, as this will lead to more effective control of gray mold disease.

Packed strawberries may be contaminated by *B. cinerea* spores on their fruits and calyx, and if there are no suitable conditions for storage, such diseases will inevitably occur. In our study, low temperature can substantially decrease incidence of disease in strawberries. Precooling temperature system was used for long distance transportation of strawberry, mushroom and so on (Kedar, 1992; Park et al., 2012; Pryke and Pringle, 2008).

Conclusion

The study set out to find if there was a correlation between the incidence of pathogen/disease in two greenhouses and a packing workplace, using environmental factors. The most important environmental factors were temperature and moisture which increased the number of spores and the overall incidence of disease. Furthermore, the inoculum source of the disease that appeared in the packing workplace originated in the cultivation fields. This conclusion suggests that to reduce or eradicate gray mold disease, measures must be put in place at the site of cultivation and not where strawberries are packed and stored.

Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Project No. 315004-5).

References

Ayerst, G. (1969) The effects of moisture and temperature on growth and spore germination in some fungi. J. Stored Prod. Res. 5(2):127-141.

Braun, P. G. and J. C. Sutton (1987) Inoculum sources of *Botrytis cinerea* in fruit rot of strawberries in Ontario. Canadian Journal of Plant Pathology. 9(1):1-5.

Burt, P. J., J. Rutter and H. Gonzales (1997) Short-distance wind dispersal of the fungal pathogens causing Sigatoka diseases in banana and plantain. Plant Patol. 46(4):451-458.

Coley-Smith, J. R., K. Verhoeff and W. R. Jarvis (1980) The biology of *Botrytis* (pp41-83). Press.

Elad, Y., B. Williamson, P. Tudzynski and N. Delen (2004) *Botrytis* spp. and diseases they cause in agricultural systems-an introduction. Botrytis: Biology, pathology and control. : 1-8.

El-ghanam, A., S. S. Ragab and S. A. Farfour (2015) Bio-suppression of strawberry fruit rot disease caused by *Botrytis cinerea*. Plant Pathology & Microbiology. S3:005.

Hirst, J. (1953) Changes in atmospheric spore content: diurnal periodicity and the effects of weather. Transaction of the British Mycological Society. 36(4):375-393.

Hwang, Y. and J. Ku (2004) Effect of high molecular weight chitosan on the quality and decay of strawberry fruits. J. Agri. Sci. 31:77-86. (in Korean)

Jarvis, W. R. (1977) *Botryotinia* and *Botrytis* species: Taxonomy, Physiology and Pathogenicity, A guide to the Literature. Monograph No. 15. Ottawa: Canada Department of Agriculture.

Kedar, A. (1992) Postharvest biology and technology-an overview (p15-20). Postharvest technology of horticultural crops. Second Edition, UC Davis Publication.

Kerssies, A. (1993) Horizontal and vertical distribution of airborne conidia of *Botrytis cinerea* in a gerbera crop grown under glass. Netherlands. Eur. J. Plant Pathol. 99(5):303-311.

Kim, D. R., C. W. Jeon and Y. S. Kwak (2015) Studies on *Botrytis cinerea* density in packing shed and gray mold incidence following storage-temperature in exported strawberry. Korean J. Pestic. SCI. 19(3): 295-300. (in Korean).

Muñoz, Z. and A. Moret (2010) Sensitivity of *Botrytis cinerea* to chitosan and acibenzolar-S-methyl. Pest Manag. Sci. 66(9):974-979.

Nam, M. H., H. S. Kim, W. K. Lee, M. L. Gleason and H. G. Kim (2011) Control efficacy of gray mold on strawberry fruits by timing of chemical and microbial fungicide applications. Kor. J. Hort. Sci. Technol. 29(2):151-155. (in Korean).

Park, J. E., H. M. Kim and S. J. Hwang (2012) Qualitative changes in precooling and storage temperatures on the postharvest management of the fruits in strawberry for export. J. Agricul. Life Sci. 46: 67-74. (in Korean).

Pasanen, A., P. Kalliokoski, P. Pasanen, M. Jantunen and A. Nevalainen (1991) Laboratory studies on the relationship between fungal growth and atmospheric temperature and humidity. Environ. Int. 17(4):225-228.

Pryke, J. and K. Pringle (2008) Postharvest disinfestation treatments for deciduous and citrus fruits of the Western Cape, South Africa: a database analysis. S. Afr. J. Sci. 104(3-4):85-89.

Rosslenbroich, H. and D. Stueble (2000) *Botrytis cinerea*-history of chemical control and novel fungicides for its management. Crop Protection. 19(8):557-561.

Segarra, G., E. Casanova, C. Borrero, M. Avilés and I. Trillas (2007) The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plant. Eur. J. Plant Pathol. 117(4):393-402.

Sistrunk, W. A. and J. R. Morris (1985) Strawberry quality: influence of cultural and environmental factors. In Evaluation of quality of fruits and vegetables (Anonymous). Springer : 217-256.

The Korean Society of Plant Pathology (2009) List of plant disease Korea. 5th ed. Seoul: Korean Society of Plant Pathology.

Tripathi, P. and N. Dubey (2004) Exploitation of natural products as an alternative strategy to control postharvest fungal rotting vegetables. Postharvest Biol. Technol. 32(3) : 235-245.