

6. Modification of pathogens

Pathogens are exposed to high chances of modification because of a short generation time and simple chromosome structure. Modification is random, but in some cases, the modulated pathogens are capable of attacking the resistance host or vice versa. The concept of “race (physiological cultivars)” comes from the same context.

Adaptation is a temporary change, and the adapted plants are supposed to be restored to the former condition when the environment changes.

7. Plant-microbe interactions

Disease resistance and susceptibility are considered to be under the control of strength of pathogen pathogenicity and host resistance until Flor proposed “gene-for gene theory” in 1956. Flor’s theory clarified the concept of race and reformed the research direction of plant disease resistance.

Development of genetic and molecular technology has been placed on confirming the hypothesis that the virulence (Vir) genes (pathogen) and the resistance (R) genes (host) are dominant or recessive, and pairing of Vir and R genes determines disease resistance. These days genetic approaches become the major stream of plant disease research, to which MPI scientists and young researchers devote themselves.

8. Control of plant diseases

Since plant disease is a biological phenomenon caused by pathogen, plant, and environment as a complex, overall understanding of each factor is necessary to protect plants from diseases. Additionally, it is important to consider “group” of plants when controlling plant disease.

Development of control substances

We should aim at generating an effective and safe disease control substance under the preferential consideration of human environment. Recently researchers conduct active studies on the biological control, which bases its principle on the competition for survival between living creatures, and they take an interest in antagonistic microorganisms.

Many chemicals, used for plant disease control, have shown to be toxic to pathogens and may be toxic to other organisms including human. Although application of chemicals is often necessary for food production, we cannot neglect to find other environmentally friendly means of controlling plant diseases.

Selection and breeding of resistance plants

Plant breeding usually intends to increase yield or produce high quality product, but generation of disease resistance plants can be the most important purpose for plant breeders. Despite production of many superior cultivars, conventional breeding costs much time but ends up with low successful rate. Therefore genetic engineering technology is applied for production of disease resistance plants, which is called as “Genetically Modified Organism (GMO)”.

Integrated pest management

Integrated pest management (IPM) is the application of not only the means to inhibit pathogen activity but also fertilization and management to raise disease tolerance as follows, cultivation of resistant cultivars, maintaining of fertile soil using organic fertilizers, the fewest application of pesticides, and elimination of inoculum.

Conclusion

All the scholars, who study plant pathology, are familiar with the content described above. Therefore, they should be fully aware of this knowledge and not hesitate to answer the questions involved.

Additionally I would like to point that plant disease research has to be carried out in a successive and organized manner, beginning from the fundamental knowledge. For example, even the research using high-tech technology will become meaningless without fundamental understanding of disease symptom or pathogen ecology.

The importance cannot be weighed on a specific research fields. For example, as imports and exports of agricultural products increase and crops become diversified, new plant diseases are occurred, thus disease diagnosis itself is very important but it also creates new subjects for disease studies. The research has to initiate from the first step, and then proceed to the conclusion in a rational way.

As the concerns of a plant pathologist, the curriculum of undergraduate can be regarded as a fundamental course of agricultural biology and that of graduate as an internship to become a plant pathologist. In some cases, a graduate student jumps to a highly specified area too early, loses the chance of internship training, therefore becomes a defective scientist. I would like to ask the students to undergo internship course to become a genuine plant pathologist.

AL-2

Academic Award

Disease Problem and Its Solutions in Apple Industry of Korea

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1. Disease problems in apple industry in Korea

As apples have long been bred only for good taste and high yield, while disease resistance has been almost completely

neglected, much more diseases occur on apple than other major crops. Moreover, due to the nature of perennial crops, eradication of diseases from the orchard is very difficult once they had

occurred. Therefore, commercial apple production is almost impossible without pesticides. In Korea, due to the frequent rain during the apple growing season, especially in the one-month of the rainy season, disease is much more serious problem than in other major apple production areas of the world. If fungicides are not used, more than 90% of the fruit might be rotten before harvest and the trees might lose almost all of their leaves.

In Korea, 34 parasitic apple diseases including three viral and two bacterial diseases have been described, but the actual occurrence of some diseases has been doubted. A survey conducted from 1991 to 1993 in Kyungpook Province revealed that 21 diseases occurred in moderately well-managed orchards. Among them, 7 diseases, rust, *Alternaria* blotch, *Massonina* blotch, white rot, bitter rot, sooty blotch and apple Valsa canker, were economically important. Especially, epidemics of white rot, bitter rot, and *Marssonina* blotch are so severe that they sometimes cause serious economic problems for the family budget of the growers. For instance, due to the severe epidemics of white rot and *Marssonina* blotch caused by the long spell of rain for 60 days from May to August in 1998, many farmers gave up the harvest.

The spray program developed by apple production cooperation recommends 16 sprays in one cropping season, two sprays before blooming and at 10-day intervals from petal fall to mid-September. In a survey of the state of chemicals used in 215 apple orchards during 1997 and 1998, the average spray frequency was 13.7 times, but only about 20% of farmers sprayed more than 16 times in a year. The more serious problem was that more than 30% of the farmers sprayed two kinds of fungicides to strengthen the control efficacy. In spite of frequent spraying, the economic loss due to white rot, bitter rot, and *Marssonina* blotch was severe.

2. Present status of the major apple diseases that require chemical spray for control and strategies for controlling.

1) Rust

The rust caused by *Gymnosporangium yamadae* Miyabe: Yamada is one of the most common disease that occurs in almost all apple growing areas in Korea. It was a serious apple disease before introduction of EBI fungicides in apple production, but it can now be easily controlled by a single spray of EBIs after petal fall.

2) *Alternaria* blotch

Alternaria blotch incited by a pathogenic strain of *Alternaria mali* Roberts (*A. alternata* (Fr.:Fr.) Keissl., apple pathotype) varies markedly in severity with different cultivars, and it has a short incubation period. The Fuji cultivar which is the leading cultivar in Korea, is moderately resistant to this disease, and defoliation has seldom been seen in the past. Recently, the high density planting orchard with M.9 rootstock has been introduced and is rapidly replanting the conventional type of orchards. The Fuji cultivar that are resistant to this disease become susceptible when it is grafted on the M.9 rootstock, and sometimes it requires special spray. The pathogen also infects the fruit, but does not cause rot, and only affects marketability because of lenticel discoloration and black spots.

The pathogen winters in dead leaves on the ground or on lesions formed on the young shoots grown in the previous year.

Primary infection occurs during the blooming period by the conidia produced on them. The lesions produced by the primary infection on the young leaves can be seen as early as around petal fall. However, large-scaled infections by the secondary infection sources usually occur in late May to early June.

In most cases, *Alternaria* blotch can be controlled simultaneously with other diseases such as white rot and *Marssonina* blotch in Korea. Even though highly effective fungicides for control of this disease have been developed, but their control efficacies against other diseases that is to be controlled simultaneously. Therefore, they should be used only for special sprays in the case of a sudden epidemic. Fortunately, as the incubation period of this fungus is very short, it is enough to spray the fungicides when epidemics are confirmed.

3) *Massonina* blotch

Marssonina blotch caused by *Diplocarpon mali* Harada & Sawamura (anamorph *Marssonina coronaria* (Ell. & J. J. Davis) J. J. Davis, syn. *M. mali* (Henn.) Ito) has recently emerged as an economically important apple disease in Korea. This disease has been known in Korea since the early period of apple cultivations, but it was not much of a problem during the period when the Bordeaux mixture was routinely used in apple growing. Most apple growers in Korea excluded the Bordeaux mixture from their spray calendar in the early 1970s along with the planting of Fuji cultivar. After the great epidemics of 1993, *Marssonina* blotch has occurred in every orchard where the fungicidal spray has not been adequate, and has caused serious damage.

The pathogen winters in the dead leaves on the ground, and primary infections are initiated by ascospores in apothecia produced on them. Even though the mature ascospores are found as early as before bloom stage, the maximum discharge is estimated to occur in early June. The primary symptoms appear in the middle of June, usually on mature leaves.

Control of this disease may be achieved through orchard sanitation to some extent but fungicidal spray is necessary. In order to block the primary infection, highly efficacious fungicides against this disease should be sprayed from late May to late June. However, as the fruit is also highly vulnerable to bitter rot at that time, the fungicides employed should also be efficacious against bitter rot. As the disease development that had been suppressed by the high temperature is resumed in late August or early September when the temperature goes down, the fungicides to be sprayed in August, at which time the control of white rot with curative fungicides is essential, should also be efficacious against this disease. Therefore, *Marssonina* blotch should also be controlled simultaneously with bitter rot and white rot.

4) White rot

White rot caused by *Botryosphaeria dothidea* (Moug) Ces. & De Not. also emerged as one of the most serious diseases with the increased planting of Fuji cultivar which is highly susceptible to this disease. At present, because the Fuji cultivar constitutes more than 70%, white rot is the most important disease. In the past, almost one half of the fungicides sprayed during the cropping season were for control of white rot.

The pathogen not only causes fruit rot but also weakens the tree by infecting the stems. On stems infected by this fungus, warts

are formed in which a large number of pycnidia and sometimes ascospores or pycnidiospores produced in the warts; the latter is more important as inoculum. The infection of fruit either by ascospores or pycnidiospores can occur as early as one or two weeks after petal fall, but symptoms develop later than mid-August.

The difficulties controlling of this disease lie in the fact that the inoculum of the fruit infection comes from the diseased stems, and that it disperses whenever it rains throughout almost the whole growing season. In the early stage of our work to fight this disease out, every effort to reduce the inoculum density or to block their dispersal was unsuccessful. However, in an experiment to examine the duration of after-infection activity of EBIs against apple white rot, an important clue was found. The infected fruit, regardless of their infection time, can be cured or at least the symptom development can be suppressed by a single application of EBIs around mid-August when the infection is almost over. Other important information on the control of this disease was obtained in an experiment to select protective fungicides to be sprayed before EBI. Several nonsystemic fungicides such as folpet and iminoctadine-triacetate and the systemic fungicide azoxystrobin showed post-infectious activity, and the incidence of disease was significantly reduced when the infected fruits were treated with them. Combining the information above, effective spray programs can be developed.

5) Bitter rot

Bitter rot which is one of the most common diseases of apple, also caused serious damage until 1960s when the Ralls Jannet and Jonathan was leading cultivars. However, since Fuji cultivar which is moderately resistant to this disease was substituted for Ralls Jannet from the beginning of the 1970s, bitter rot has been almost neglected in the spray program. The incidence of bitter rot became gradually more noticeable in the late 1990s usually on early or mid-season varieties. Since around 2000, bitter rot has caused significant damage not only to susceptible varieties but also to Fuji cultivar which is resistant to this disease. In some cases, the damage by this disease is almost equal to that of white rot on Fuji cultivar.

The main inoculum source of primary infection of this disease is the spores produced on the mycelia surviving in wound on branch or twigs. Another important inoculum source is the spores produced on wild black locust (*Robinia pseudo-acacia* L.) near the orchard.

The control of this disease almost solely depends on regular spraying of protective fungicides, and no fungicides of eradication efficacy are available at present. Two facts make it more difficult to control bitter rot in Korea. One is that most orchards grow a mixed culture type of Fuji and other early- or mid-season varieties which show opposite dispositions in the resistance against white rot and bitter rot. The second fact is that few fungicides can control white rot and bitter rot simultaneously with equal efficiency. Fuji cultivar is highly susceptible to white rot but highly resistant to bitter rot, and most of early- or mid-season varieties *visa versa*. Fungicides such as iminoctadine-triacetate and tebuconazole that are highly efficacious against white rot show poor performance against bitter rot. Accordingly, in orchards where Fuji and other bitter rot susceptible varieties are mixed, selection of fungicides is

pseudothecia are produced. The infection source of fruit rot is the very difficult, especially since the time of infection in both diseases is roughly the same.

3. Development of spray program with 15-day interval from petal fall to late August.

Because the possible infection period of the 5 diseases that should be controlled by chemical means are roughly the same except for rust, it might be beneficial to control them simultaneously. Among them, the highest priority should be paid to white rot in Fuji cultivar and to bitter rot in most of mid-season varieties. At the beginning of our study, control of white rot was our main concern. If the white rot is properly managed, other diseases are of less importance, since the bitter rot was no problem at that time at least on Fuji cultivar which accounts to 78% of apples in Korea. Marssonina blotch can easily be managed by a single application of an effective fungicide per month, if the primary infection is effectively blocked. Although *Alternaria* blotch occurs every year, it rarely becomes serious, and can also easily be managed. It may only be necessary to control the disease by a special spray of specific fungicides in the case of severe epidemics.

On the basis of this information and the results of the experiment on the properties of fungicides against those diseases, a spray program in which fungicides were scheduled to spray at 15-day intervals from petal fall to late August (15-day program) could be developed. As apples are cross-pollinating, several different varieties are usually planted in most of Korean apple orchard in a single plot to promote fertilization. Orchards were classified into four types on the basis of susceptibility to white rot and bitter rot, and spray programs were developed in accordance with the composition of the apple cultivars.

4. Development of a 25-day interval spray program from petal fall to late August

In the course of the experiment to develop the spray programs, two facts that suggest further reduction of fungicides were revealed. Infection by two fruit diseases occurs during most of the growing season, but symptoms begin to develop only after the termination of chemical spraying. Therefore, the control efficacies of the fungicides sprayed during the growing season are collectively expressed at harvest, and the efficiencies of individual fungicides on the control of the diseases are difficult to assess. If they can be assessed, improvement of a spray program can easily be done.

As an effort to assess them, 7 plots, equivalent to the number of spray time during the possible infection period of both diseases, were sprayed with fungicides according to a spray program of 15-day intervals. At each spray time, the fungicide to be sprayed at that time was omitted in one plot. The control efficiencies of individual fungicides were assessed by comparing the disease incidence and infection frequency in each fungicide-omitted plot with those of the completely sprayed plot. When the disease incidence in the fungicide-omitted plot was higher than the completely sprayed plot, the omitted fungicide was regarded having positively contributed to the control of the disease; when the former was lower than the latter, the omitted fungicide was regarded as negatively contributed. When the incidences were not

significantly different from each other, they were regarded as not-contributed.

Using these standards, the efficiencies of individual fungicides were assessed. The results varied considerably according to the year, reflecting the yearly differences in the intensity and time of infection. However, the number of positively contributed fungicides was usually less than that of the negative or not-contributed group. This fact strongly suggests that fungicides can be reduced through extension of the spray interval in the program.

Another fact suggesting the reduction of fungicide was obtained in an experiment to detect the maximum duration of the protective activity of the fungicides constituting the spray program against white rot. The fungicides were sprayed in late June when active infections occur, and the fruits were periodically bagged with two-layered fruit bags for 45 days from the spraying. The duration of protective activity was assessed by the disease incidence shown by the fruit bagged on different days. High protective activity of several fungicides including azoxystrobin and dithianon, was maintained for 45 days, and 30 days in iminoctadine-triacetate. This also suggested that the spray interval could be extended more than 15-days.

Given the two facts above, spray programs of different spray intervals, 15, 20, 25 day, were made with same fungicides. However, as the spray intervals were extended the spray times decreased, and one and two fungicides in the 15-day spray program were excluded in the 20- and 25-day program, respectively. They were subjected to a pilot test on Fuji cultivar,

and the control efficiencies against various diseases were examined. The tests were conducted for two years with slightly different programs. Even though the results were slightly varied according to the year and the program, the control efficiencies of 20- and 25-day programs were superior to those of 15-day program, or not differ with them. Upon those experimental results, several 25-day programs varied in the kind of fungicides and spray sequences were subjected to a pilot test. Control efficiencies were quite different among the programs, but some of them turned out to be highly promising. One program that showed good control efficiencies on white rot, bitter rot, and Marssonina blotch was subjected to an actual orchard test at 16 and 54 orchards in 2002 and 2003, respectively. The results were quite satisfactory. The diseases were effectively managed in most of orchards but slight damage by white rot or Marssonina blotch was recognized in a small number of orchards. Especially in 2003, in spite of the adverse climatic condition in which 58 days of rain during the vulnerable season to various diseases from May to August, good control was obtained in most of the orchards. By 2004, several hundred of farmers have already adopted the 25-day program for Fuji cultivar, but most of farmers still hesitate because of possible failure.

At present, the 25-day program can be used without any concerns with Fuji, Tsugaru and a few mid-season varieties that are moderately resistant to bitter rot. Efforts to develop the programs for cultivars susceptible to bitter rot are on going and will be the subject of our future research.

AL-3

Young Plant Pathologist Award

A Platform for Functional Genomics of Chili Pepper Defense against Pathogen

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Large-scale single-pass sequencing of cDNA libraries and microarray analysis have proven to be useful tools for discovering new genes and studying gene expression. As a first step in elucidating the defense mechanisms in hot pepper plants, a total of 30,000 expressed sequence tags (ESTs) were generated and constructed as a public database. The cDNA microarray which contain 4,815 independent pepper clones is developed and gene expression analysis identified 613 hot pepper genes that were transcriptionally responsive to the non-host soybean pustule pathogen *Xanthomonas axonopodis* pv. *glycines* (*Xag*). Several functional types of genes, including those involved in cell wall modification/biosynthesis, transport, signaling pathways and divergent defense reactions, were induced at the early stage of *Xag* infiltration. In contrast, genes encoding proteins that are involved

in photosynthesis, carbohydrate metabolism and the synthesis of chloroplast biogenetic proteins were down-regulated at the late stage of *Xag* infiltration. These expression profiles share common features with the expression profiles elicited by other stresses, such as fungal challenge, wounding, cold, drought and high salinity. We also identified several novel transcription factors that may be specifically involved in the defense reaction of the hot pepper. All the gene expression data were constructed as a public database and opened to research community. Functional analyses of selected novel genes are underway and part of it will be presented in the symposium. This study is the first report of large-scale sequencing and transcriptome analysis of the hot pepper plant species.