日本におけるオゾンによる農作物被害軽減のための農業対策技術

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Agricultural Countermeasures for Avoiding Crop Injury from Ozone in Japan

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Abstract

Photochemical oxidant pollution and related occurrences of plant injury have occurred in Japan every year since 1969-1970. Crop injury by photochemical oxidants (mainly ozone) is directly connected with agricultural economic loss. Especially, the marketable values of damaged leafy vegetables are greatly reduced, causing drastic economic losses. Five methods of agriculture technology for avoiding damage resulting from ozone are considered: (1) inherited resistance properties of crops; (2) fertilizer management; (3) treatment with specific chemicals; (4) biotechnological activation of defense genes; and (5) in-facilities cultivation. However, until now these methods only offer a temporary countermeasure, providing limited protection and are useful only against occurrence of mild injuries.

Key words: Air pollution, Countermeasures, Foliar injury, Ozone, Photochemical oxidants.

1. History of Plant Injury by Air Pollution and Current Research Status in Japan

Plant injury by air pollution in Japan can be traced along the following historical path: (1) dieback of forest trees and reduced growth and yield of crops near mines and metal refineries, caused by sulfur dioxide (SO_2) emissions from around 1610; (2) plant injury, mainly to roadside trees by coal produced smoke (soot and SO_2), rising from the numerous chimneys of small factories in the Osaka and Tokyo areas between 1900 and 1950; (3) injury of mandarin orange, pear and rice crops due to SO_2 located around the industrial complexes of large factory groups during the period of high economic growth (1955 to 1970) after World War II, in which the major fuel source switched from coal to petroleum and natural gas; (4) injury of agricultural crops and park and roadside trees in big city areas such as Tokyo, Nagoya and Osaka, caused by photochemical oxidants, which are produced in the atmosphere by complex photochemical reactions involving nitrogen oxides and hydrocarbons, and are understood to include ozone (O_3), peroxyacetyl nitrate ($CH_3COOONO_2$), hydrogen peroxide (H_2O_2) and others, due to the rapid increase in population and transportation, in addition to industrial growth after 1970; (5) symptoms of tree crown damage in mature Japanese cedar, *Cryptomeria japonica,* in the flatlands since the late 1960s; and (6) recent forest decline in various mountain areas.

At present, the main target of plant effect research by air pollution in Japan as well as in Europe and North America is to clarify the causes and/or mechanisms of forest decline. Air pollution effects scientists have conducted experimental studies and field surveys on the effects of ozone (about 90% of all photochemical oxidants found in the air), simulated acid rain and soil acidification on Japanese forest tree species for the past decade (*e.g.* Maruta *et al.,* 1999; Kohno and Matsumura, 1999; Izuta *et al.,* 2001a, 2001b). In addition, research on elucidation of injury mechanism caused by ozone and production of ozone tolerant transgenic plants by using recent molecular biology has also actively pursued (*e.g.* Sakaki *et al.,* 1994; Kubo *et al.,* 1995; Nakajima *et al.,* 2002). In the meantime, research related to threshold concentrations of ozone for agricultural

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crops and research of countermeasures for avoiding crop injury from ozone has not been emphasized in Japan since the late 1980's.

In recent studies, high ozone concentrations have been reported in Asia's major cities such as Taipei (Liu *et al.,* 1994), Hong Kong (Wang *et al.,* 1998), Shanghai (Xu *et al.,* 1999) and Seoul (Ghim and Chang, 2000). In fact, foliar injury of agricultural crops caused by ozone has already been observed in Taiwan (Sun, 1996). In China, reduction of crop yields will be anticipated in the future due to elevated ozone concentrations, and productivity may already be affected for some crops (Chameides *et al.,* 1999; Aunan *et al.,* 2000). It may be possible to utilize various technologies developed in Japan such as use of indicator plants to detect or monitor ozone and mitigation technology for crop damage, in other Asian countries. In addition, although there is a large fluctuation in numbers of photochemical smog warnings from year to year (Fig. 1), which are issued when the hourly average of photochemical oxidant concentration exceeds 120 ppb, photochemical oxidant concentrations over Japan tended to increase during the past 15 years (from April 1985 to March 2000) at a rate of 0.33 ppb per year (1.1% per year) (Ohara and Sakata, 2003). Therefore, severe crop damage that was frequently observed in 1970's may occur again. I review agricultural countermeasures for avoiding crop loss from ozone, and show the limit to their effectiveness.

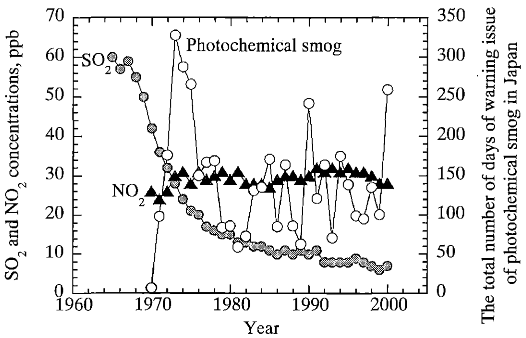

Fig. 1. Changes in annual mean concentrations of SO_2 at 13 air pollution monitoring stations (4 in Tokyo, 4 in Yokohama, 3 in Kawasaki, 1 in Yokkaichi and 1 in Osaka) and NO_2 at 14 air pollution monitoring stations (1 in Chiba, 6 in Tokyo, 1 in Kawasaki, 1 in Nagoya, 1 in Osaka, 1 in Amagasaki, 1 in Kurashiki, 1 in Matsue and 1 in Kitakyushu) continuously operated since 1965 and the total number of days when photochemical smog warnings were issued in Japan (Data from Air Pollution in FY 2001 in Japan).

Photochemical smog warnings are issued when the hourly average of photochemical oxidants concentration exceeds 0.12 ppm. Taking administrative divisions as 1 unit, the announcement frequency of the photochemical smog warning totaled each prefectural announcement number.

2. The Present State of Air Pollution in Japan

At present, the adoption of low sulfur containing fuel and the installation of flue gas desulfurization equipment have greatly reduced SO_2 air pollution (Fig. 1) and the occurrence of related plant injury is now no longer apparent in Japan. On the other hand, photochemical oxidant pollution has still occurred every year (Fig. 1) and related plant injury has continued since it was first recognized around the Tokyo area in 1969–1970. Moreover, the annual average pH of precipitation (rain and snow) at 47 monitoring stations in 2000 ranged from 4.5 to 6.2 with a mean value of 4.8 in Japan (Acid Deposition Survey, Ministry of the Environment Government of Japan, 2002). This annual average pH did not change significantly in the past 15 years (Acid Deposition Survey, Ministry of the Environment Government of Japan, 2002). Until now there have been almost no reports of visible injury or yield loss of agricultural crops in the field due to acid rain except for bleaching spots on petals of morning glory in Japan. Therefore, the current ambient levels of acid rain in Japan may not affect the growth or yield of crops. On the other hand, the effects of acid rain on forests are not yet understood.

The problem of crop injury as a result of air pollution is important because it is directly connected with agricultural economic loss. Leaf vegetables such as spinach, welsh onion and *Cryptotaenia japonica* ("Mitsuba") are especially sensitive to ozone. For example, exposure to ambient ozone at a level of 120 ppb for a few hours frequently produces severe injuries on the leaves of green vegetables, with symptoms appearing as bleached spots and bifacial necrotic lesions (Nouchi *et al.,* 1988). Crops with damaged leaves have greatly reduced marketable values, resulting in dramatic economic losses. It is also possible that a reduction of growth or yield can occur without foliar injury, when crops are exposed to a relatively low ozone concentration (50–60 ppb) for long periods (*e.g.* Kobayashi *et al.,* 1995).

3. Mitigation Technology against Crop Damage Caused by Ozone

The primary measure effective against plant injury by air pollution is to reduce the discharge of air pollutants. For example, in Japan, visible injury to the leaf buds and flowers of orchard pear trees and to the leaf buds of roadside ginkgo, Japanese zelkova and plane trees occurred in the Tokyo-Chiba littoral industrial area during 1965–1966 and was caused by SO_2 emissions (Shiratori, 1978). Severe damage to pear trees was particularly common, including not only necrotic legions on leaves and defoliation, but also the death of flowers and damage to young fruits. To prevent such damage, the Chiba prefectural government issued pear injury warnings when the average hourly SO_2 concentration exceeded 150 ppb, or when the weather conditions during the flowering and young fruit stages made damage particularly likely. When warnings had been issued, local factories were requested to suppress SO_2 emissions by switching to low sulfur containing fuel. In addition, the prefectural government guided the improvement of pear cultivation methods and encouraged conversions to the planting of alternative crops. Following these measures, pear injury was eliminated (Shiratori, 1978).

In the meantime, there has been no improvement in the level of pollution caused by photochemical oxidants, and ozone-induced injuries on morning glory and taro and PAN-induced injuries to petunia have been reported every year (Department of Air Pollution, Environmental Protection Measures Promotion Headquarters, Kanto District Governors Association, 1998). Even though ozone and PAN are photochemical oxidants, PAN causes much less injury in the field than ozone. At present, the most widespread phytotoxic air pollutant that exerts the greatest effect on agricultural crops and terrestrial ecosystem is ozone. Although national and prefecture governments have attempted to bring about the reduction of photochemical oxidant pollution, by for example, introducing various countermeasures for the reduction of nitrogen oxides (one of the main contributors) at the source, significant progress has not yet been made (Fig. 1). During high-temperature combustion, nitrogen combines directly with oxygen in the air, producing nitrogen oxides. In addition, the absolute number of automobiles and diesel engined vehicles, which discharge large quantities of NO_x, has continued to increase. Since it is difficult to undertake source countermeasures to control NO_x emissions because of complex, multiple factors, and farmers on the receiving end of the damage must therefore personally attempt to limit the spoilage to crops.

The extent of plant damage by ozone is determined by the pollutant dose (concentration × time) (Larsen and Heck, 1976; Nouchi, 1979). However, the extent of injury also varies greatly among different crops: factors such as genetic differences (both within and between species and varieties), physiological attributes (for example, growth stage) and environmental conditions such as radiation, temperature, humidity, soil moisture and fertilizer levels, all exert an influence (Guderian, 1985). In general, foliar injury caused by ozone occurs most often during the vigorous initial to middle growth stages under moderate growth conditions, which are free from environmental sources of stress, such as water stress. By utilizing these properties in crops, the following five agricultural techniques have been proposed to avoid plant damage resulting from ozone.

3.1 Utilizing inherited resistance properties of crops

The extent of plant injury caused by ozone differs greatly among species and even among cultivars (Nouchi *et al.,* 1988). For example, acute foliar injuries on very sensitive plants such as morning glory, spinach, radish, Chinese cabbage (*Santosai*) Chinese rape (*Brassica campestris* L. var. komatsuna), tobacco, bush bean and welsh onion are often observed when exposed to daily maximum photochemical oxidant concentrations of 80–100 ppb, whereas similar injuries to tomato, rice, lettuce, Great radish, leaf beet, corn and peanut occur when exposed to concentrations of 100–120 ppb. In addition, ozone or PAN damage to cabbage, cauliflower, pumpkin, melon, strawberry, onion and gladiolus have never been observed in the field, suggesting that these species are very resistant to ozone or PAN (Nouchi *et al.,* 1988).

In spinach, field susceptibility to ambient ozone differs markedly among cultivars (Fig. 2). Oriental

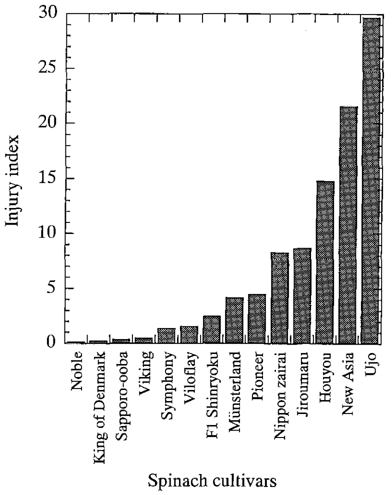

Fig. 2. Leaf injury of spinach cultivars by ambient ozone in the field in Tachikawa, Tokyo on May 12–17, 1978 (revised from Kuno, 1988). Each bar represents the mean of 24 plants. Seedling date : March 24, Daily maximum concentration of photochemical oxidants : 120 ppb on May 12, 80 ppb on May 13, 130 ppb on May 14, 140 ppb on May 16 and 120 ppb on May 17.

Injury index $= (\Sigma nl) \times 100/5L$

where n : grades of injury from 0 to 5 ; 0 : 0% (no injury), 1 : less than 25% (percentage of damaged area of each leaf), 2 : 25% to less than 50%, 3 : 50% to less than 75%, 4 : 75% to less than 100% and 5 : 100%, l : numbers of leaves injured or not injured by ozone, L : number of all leaves of a plant.

cultivars with leaves that are abundantly notched or thin, for example, "Ujo", "Houyou", "Jiroumaru", "Nippon zairai", *etc.* are sensitive to ozone (Kuno, 1988). On the other hand, Occidental cultivars with leaves that are less abundantly notched, thick or round, for example, "Vilofly", "King of Denmark", "Nobel", *etc.* are resistant to ozone (Kuno, 1988). Similarly in varieties of potato, it is known that "Danshakuimo" and "Toyoshiro" are sensitive to ozone, but that "Waseshiro" is resistant (Matsumaru and Takasaki, 1989). During periods when photochemical oxidant pollution frequently occurs, it is necessary to stop planting highly sensitive leaf vegetables such as spinach, or to choose resistant cultivars.

New highly pollution resistant cultivars might be developed using both hybridization breeding and recently developed biotechnology techniques. Reciprocal crossings of resistant Occidental cultivars, such as "Vilofly", and sensitive Oriental cultivars, such as "Ujo", have shown that ozone resistance is determined by a female character (Kuno, 1988). The hybrids were moderately sensitive, for example, "F1 Synryoku" ("Nippon-zairai" × "King of Denmark"), "Viking" ("King of Denmark" × "Viroflay"), "New Asia" ("Ujo" × "Sapporo-ooba") and "Houyou" ("Nippon-zairai" × "Münsterland"). These results suggest that an inherited character related to ozone resistance of spinach was inherited from female cytoplasm (Kuno, 1988), and that the production of hybrid cultivars of stronger resistance than the parental phenotype is a difficult objective.

3.2 Damage reduction by fertilizer management

It is known that the responses of plants to ozone greatly differ according to the growing environments of crops and the nutritional states of the soils in which they are grown. Fertilizer management technology, such as the application of fertilizers, is a method for reducing damage by maintaining the conditions under which the resistance of crops to ozone is highest.

Although there have been many studies relating to the effect of fertilization on ozone damage, their results are contradictory (Guderian, 1985). For example, tobacco plants grown at excess or deficiency of nitrogen were more severely injured by ozone than plants which received the optimal levels (MacDowall, 1965). By contrast, ozone injury of tobacco was most severe on plants grown under optimum soil nitrogen levels, and damage tended to decrease when plants were grown under either deficient or excessive nitrogen levels (Leone *et al.*, 1966). However, according to a literature survey by Kuno and Arai (2002) of several Japanese studies related to fertilizer effects on foliar injury of plants to ozone in the fields or exposure experiments, foliar injury of some plants such as spinach, radish and morning glory but not rice tended to decrease with increasing nitrogen, phosphorus, and potassium levels. In the case of rice plants, foliar injury by ozone increased with increasing amount applied nitrogen.

Since ozone injury of spinach tends to decrease when the supplies of potassium, phosphorous and nitrogen increase, application of fertilizer is one effective method for avoiding injury caused by ozone. In fact, Kuno (1988) confirmed that spinach plants supplied with two- and four-times normal levels of nitrogen, phosphorus, and potassium showed less foliar injury compared with those applied standard levels. Although the reason why crop tolerance to ozone is enhanced by an excessive supply of fertilizers is not clear, there are some reports that the increase of resistance to ozone brought about by the excessive nutrient supply can be attributed to an increase in enzyme activities and the amounts of redox compounds in the defense system to active oxygen (Kuno, 1989a, b). The basal application of excessive supplies of fertilizers leads to salinization of the soil. It is therefore recommended that fertilizer management, which stimulates normal initial growth by an adequate basal application and subsequently accelerates growth by the addition of a small top dressing, should be carried out as a practical method for reducing injury due to ozone (Kuno, 1988; Kuno and Arai, 2002).

3.3 Damage reduction by treatment with specific chemicals

Two approaches using physiological and treatment with specific chemicals have been applied in the reduction of damage to crops caused by ozone. The first approach is the physiological suppression of the absorption of ozone. For example, abscisic acid (ABA), one of the plant hormones causing stomatal closure, appears to reduce injury by decreasing the uptake of ozone into the leaves. The second approach is to enhance the resistance of plants to ozone by using chemicals.

It is reported that there are strong relationships between cultivar-specific differences in the ozone sensitivity of rice plants and their endogenous ABA contents (Jeong *et al.,* 1980). Furthermore, the application of ABA into the soil (50 ml of 0.1 ppm, 1.0 ppm and 10 ppm solutions were added to the soil before ozone exposure) and foliar spray (50 ml of 0.1 ppm, 1.0 ppm and 10 ppm solutions were sprayed onto leaves before ozone exposure) of rice plants reduced visible injury due to ozone exposure (0.20 ppm for 5 h) (Ohta *et al.,* 1989).

Benomyl, a pest control agent of mold, and benzimidazole, a decomposition product of benomyl, are also excellent chemicals for reducing ozone injury (Miller and Taylor, 1970; Evans, 1971). In addition, it has been proven that piperonyl butoxide, a derivative compound of benzimidazole that is used as a pyrethrine synergist in the insecticide, is an even more powerful chemical agent against ozone-induced damage (Fukuda *et al.,* 1975). A foliar spraying of 0.1% piperonyl butoxide solution suppressed visible injury due to ozone by 70–100%, and its effectiveness persisted for more than 10 days (Fukuda *et al.,* 1975). Because benomyl and benzimidazole maintain the function and content of chlorophyll, it is thought that the physiological processes connected to chlorophyll decomposition are related to the reduction of ozone injury. For example, it is known that superoxide (O_2^-) decomposes chlorophyll. It therefore seems that the action of the O_2^- scavenging behavior of these chemicals is related to the reduction of ozone injury. In addition, the most probable mechanism of protection by piperonyl butoxide is considered to be the inhibition of the ozone-induced formation of lipid peroxides (Koiwai *et al.,* 1977). Many investigators have suggested that ozone injury could be caused by the destruction of the semipermeability of cellular membranes resulting from the peroxidation of unsaturated lipids.

In 1978, a chemical component called EDU (N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea), which has a residual toxicity in plants and is not currently marketed, was shown to offer protection against ozone damage (Carnahan *et al.,* 1978). Because EDU can almost completely prevent ozone injury, it is used in field studies of the distribution of leaf damage over wide areas and in assessing crop loss due to ozone (Brennan *et al.,* 1990; Tonneijck and Van Dijk, 1997). EDU is effective both as a soil treatment (100–500 ppm) and foliar spray (500–1,000 ppm). As a soil treatment, EDU retains its effectiveness for a very long duration, if it is applied every two or three weeks. Several studies have investigated EDU-induced changes in the activity of antioxidant enzymes or in levels of metabolites. The results of these studies have been contradicted. Lee and Bennett (1982) reported that EDU increased activity of SOD, which along with other enzymes such as catalase scavenges the toxic free radicals produced when ozone interacts with plant tissue. On the other hand, other workers (Chanway and Runeckles, 1984; Pitcher *et al.,* 1992) did not detect any change of SOD activities in bean plants after EDU application. The mechanisms underlying EDU-induced ozone protection thus remain unclear.

The high price of agricultural chemicals, coupled with concerns about their residual toxicity in food crops, means that there is currently no possibility that the application of chemicals to crops will gain favor as a realistic technique for controlling damage caused by ozone.

3.4 Activation of defense genes using biotechnology methods

To produce strongly resistant transgenic plants, the mechanism by which injuries occur must be clarified. It is thought that foliar injury by NO_2 is caused by the accumulation of NO_2^- with a strong toxicity in cells (Shimazaki *et al.,* 1992). Nitrite reductase, which reduces NO_2^- to ammonium ion (NH_4^+), plays an important role in damage resistance. The development of a plant that is tolerant against NO_2 is currently being attempted by introducing a gene with high activity for the production of nitrite reductase (Morikawa *et al.,* 2002). In the meantime, it is considered that injury by SO_2 or ozone is primarily caused by strongly cytotoxic active oxygen species that are generated in cells under oxidative stress (Runeckles and Chevone, 1991). Therefore, the production of a plant with strong resistance to SO_2 and ozone has also been attempted by introducing a gene expressing high activities of defense enzymes against active oxygen species such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) in the ascorbate/glutathione cycle (Aono *et al.,* 1998). However, although transgenic tobacco plants with elevated activity of SOD in chloroplasts showed decreased injury against paraquat, an O_2^--generating herbicide, they did not show any increase in resistance to ozone. Similarly, although transgenic tobacco plants with increased GR activity in the cytoplasm or chloroplast stroma exhibited resistance to both paraquat and SO_2, there was no noticeable difference between transgenic and normal tobacco plants in the degree of injury caused by ozone (Aono *et al.,* 1991, 1993). Thus, there are only a few studies showing that transgenic plants overexpressing single antioxidative enzymes confer enhanced ozone resistance (Van Camp *et al.,* 1994; Pitcher and Zilinskas, 1996). Currently, the concept is emerging that elevated activities for more than one antioxidant enzyme such as SOD, APX and GR are necessary to mediate better ozone tolerance. In fact, it has been reported that the photosynthesis of tobacco plants simultaneously overexpressing elevated activities of APX and GR is better protected from ozone-induced reductions than in plants overexpressing only one or neither of these enzymes (Aono *et al.,* 1998).

Gene recombination technology, which enables rapidly metabolized contributors to the process of cell injury and elevated scavenging enzyme activities, might provide a powerful technique for producing plants that are tolerant to air pollutants. However, this is a developing area of research, and further study is required to obtain transgenic plants of practical use in the preservation of both agriculture and the environment.

3.5 Damage reduction by in-facilities cultivation using covering materials

In-facilities cultivation technology aims to improve the productivity and quality of crops by controlling environmental conditions such as meteorology and soil, using greenhouses, tunnels, and other such methods. A soft film is the most frequently used of the various covering materials available, and polyvinyl chloride film (PVC film) and polyolefin film (PO film) are the most common materials. Recently, the use of permeable non-woven fabric (cloth-like adherend of single fibers such as polyester) has increased notably. Although the light transmittance of non-woven fabric is lower than in transparent materials such as PVC film and PO film, moisture permeability and hygroscopicity are high, making it suitable for use as a floating row cover material. A method for preventing ozone injury by using this non-woven fabric as a covering material for plastic houses, tunnel cultivation and floating row covers has been proposed.

Ozone concentrations in tunnel cultivation using non-woven fabric are reduced by approximately 70% of those in the ambient air (Matsumaru, 1994). It is thought that the lowered ozone concentration in the tunnel is due to the shielding effect provided by the covering, and from the physical decomposition of the non-woven fabric itself. Ozone injury to spinach grown in tunnels of non-woven fabric was almost absent; spinach cultivated in an adjacent open field showed signs of considerable damage (Matsumaru, 1994). The effect of this cultivation method in reducing ozone injury is thus clearly recognized. Similarly, it was observed that ozone injury to tomato plants decreased in a glasshouse whose ventilation windows on both sides were covered with non-woven fabric, compared with the damage observed to plants growing in an open field (Matsumaru, 1994). Because the method of in-facilities cultivation is being increasingly used throughout the world, this simple, easily adapted technology offers potential in the efforts to reduce ozone damage.

4. Conclusion

The primary measure required against plant injury by air pollution including ozone is to reduce the discharge of air pollutants. However, it is difficult to undertake strengthening source countermeasures, and farmers on the receiving end of the damage must therefore personally attempt to limit the spoilage to crops. As realistic countermeasure techniques, the following three methods are recommended: (1) utilizing tolerant species and cultivars against ozone; (2) fertilizer management technology, such as the application of fertilizers, by maintaining the conditions under which the resistance of crops to ozone is highest; and (3) in-facilities cultivation technology using greenhouses and tunnels which physically decompose ozone by covering materials. However, until now these methods only offer a temporary countermeasure, providing limited protection and are useful only against occurrence of mild injuries.

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日本におけるオゾンによる農作物被害軽減のための農業対策技術

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要　約

日本では，光化学オキシダント汚染は1969年あるいは1970年以来，毎年発生しており，それに伴う植物被害も継続的に生じている。光化学オキシダント（主成分はオゾン）による農作物被害は，農家にとって直接的な経済的減収を引き起こす。特に葉被害を受けると葉菜類の市場価格は大きく低下し，極めて大きな経済的な減収となる。農作物の本質的な性質を利用することにより，大気汚染被害を軽減する農業技術として，(1) 農作物が持つ遺伝的な抵抗性，(2) 肥料管理，(3) 特殊化学物質処理，(4) バイオテクノロジーによる防御遺伝子の活性化および (5) 施設栽培の5つの方法が提案されている。しかしながら，今のところ，これらの方法は激甚な被害には対応できず，被害が軽いような汚染に対してのみ有効な一時的な対策である。

キーワード：オゾン，光化学オキシダント，大気汚染，対策，葉被害