Biological and biochemical studies on mode of action of the herbicide imazosulfuron for use in rice paddy field

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BIOLOGICAL AND BIOCHEMICAL STUDIES ON MODE OF ACTION OF THE HERBICIDE IMAZOSULFURON FOR USE IN RICE PADDY FIELD

水稲用除草剤イマゾスルフロンの作用機構に関する生物学および生物化学的研究

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Contents

ABBREVIATIONS

ALS	acetolactate synthase
CYPDI	*Cyperus difformis* L.
CYPSE	*Cyperus serotinus* Rottb.
ECHOR	*Echinochloa oryzicola* Vasing.
ELOKU	*Eleocharis kuroguwai* Ohwi
FAD	flavine adenine dinucleotide
LIDPY	*Lindernia procumbens* (Krock.) Borbas
MOOVA	*Monochoria vaginalis* (Burm. f.) Presl var. *plantaginea* (Roxb.) Solms-Laub.
OENJA	*Oenanthe javanica* (Blume) DC.
ORYSA	*Oryza sativa* L. cv. Nipponbare
PISSA	*Pisum sativum* L.
PTMDI	*Potamogeton distinctus* A. Benn.
ROTIN	*Rotala indica* (Willd.) Koehne var. *uliginosa* (Miq.) Koehne
SAGPY	*Sagittaria pygmaea* Miq.
SAGTR	*Sagittaria trifolia* L.
SCPJO	*Scirpus juncoides* Roxb. var. *ohwianus* T. Koyama
SCPPL	*Scirpus planiculmis* Fr. Schm.
TPP	thiamine-pyrophosphate
TRIAE	*Triticum aestivum* L.

CHAPTER 1

INTRODUCTION

Reflecting a shortage of arable land, Japanese agriculture is necessarily highly intensive and makes extensive use of technology and capital investment. The area available for cultivation, moreover, is shrinking as the demand for residential and industrial uses increases. As the Japanese diet becomes more diversified, the consumption of rice has declined, with increased demands for livestock products, fats and oils requiring substantial imports of food products. Part of the decline in Japan's food self-sufficiency has been due to the government's easing of restrictions on food imports under the World Trade Organization's (WTO) General Agreement on Tariffs and Trade (GATT). In the Uruguay Round of the WTO/GATT conference, Japan agreed to convert some non-tariff agricultural import measures to tariffs, which will guarantee a certain minimum access for imports. Rice was exempted from tariffication, as long as Japan increased minimum market access to 4% in 1995 and then later to 8%. To implement this commitment on minimum access, the government abolished the Food Control Law and established the New Staple Food Law in 1995. The new law effectively permitted the free marketing of rice and has resulted in a downward pressure on rice prices. As a result of the changing agricultural climate, it is strongly requested to develop labor-saving and complete weed control technology in the paddy fields on the basis of knowledge concerning chemical herbicide utility.

Over the last few decades, moreover, there has been an increasing demand for environmental and consumer safety. This in turn has led to a rise in the number and chemical complexity of compounds which must be evaluated in order to identify a single molecule that fulfils the ecotoxicological and environmental requirements of a modern herbicide. The discovery process of a new herbicide has become risky and expensive business and agrochemical research and development has evolved into a high-tech industry where success depends on a multidisciplinary approach. Agrochemical companies are faced with increasingly stringent registration requirement and escalating costs of discovering and developing novel herbicides. The more rational the approach, based on a better understanding of the fundamental properties which determine the overall effectiveness of a compound, the greater the opportunities for saving both time and money. Furthermore, information in the behavior of a herbicide using a multidisciplinary technique have paved the way toward new and exiting herbicide discovery approaches.

Technical Advancement by Herbicides in Weed Management of Paddy Fields in Japan

Weeding used to occupy an extremely important position in crop cultivation, as it was once said that agriculture is a perpetual struggle against weeds. Before 1950, when there was no herbicide available, weeds were removed by hands and/or man-power rotary cultivator which were repeated four or more times in one crop season, spending over 506 man-hours/ha (Fig. 1.1). Manual weeding in hot wet summer was strongly hard work for farmers. 2, 4-D was introduced to practical use in 1949 and proved its value by significant herbicidal action and this success accelerated development and introduction of new herbicides so rapidly. Manual weeding was reduced year by year with the extension of herbicide use and the conventional herbicides were replaced by new, more effective ones. Then the total weeding hour including herbicide application practice has shrunken to 20 hours/ha in 1992. By the way, herbicide cost in 1992 calculates to labor-cost of 25 man-hours/ha, and combining these two figures gives 45 man-hours/ha equivalent for total weeding cost [1]. This means less than 10% of 1949 figures (Fig. 1.1). Saving in weeding labor cost is

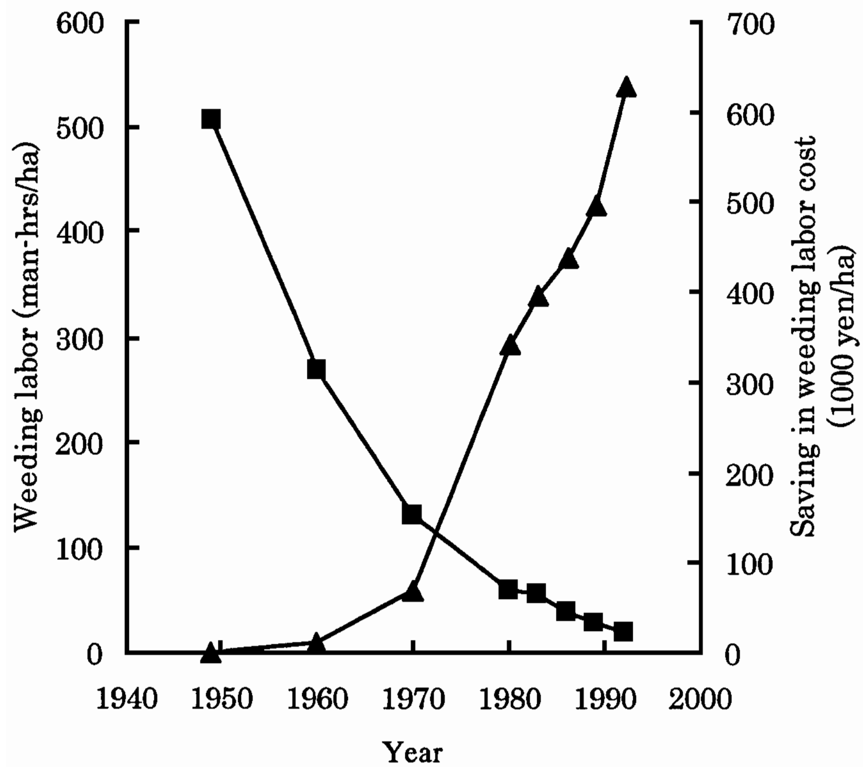


Fig. 1.1 Change of weeding labor and saving in weeding cost in Japanese paddy fields (From the statistics of MAFF and JAPR)

629,000 yen/ha. This situation results from the development of new active ingredients and the improvement of formulations as paddy rice herbicides, as well as from the rational dissemination of these herbicides.

Main weeds in Japanese paddy fields are listed in Table 1.1 [2]. However, noxious weed species in paddy fields had changed by year and by decade in Japan after introducing herbicides for rice (Table 1.2) [2]. Before introducing herbicides, *Echinochloa oryzicola*, annual broadleaves such as *Monochoria vaginalis* and some other species were noxious there under the weed control practice by hand and rotary weeder. After introducing 2,4-D and MCPA for broadleaves weed controlled in the 1950's, *E. oryzicola* became the dominant weed and caused the serious problem in paddy fields to eradicate by hand before reducing rice yields. By extending pentachlorophenol (PCP), nitrofen and chlornitrofen (CNP) in the 1960's, which were effective to kill *E. oryzicola* and other annual weeds at germination, *Eleocharis acicularis* became very serious there, because the growth and the yield of rice clearly inhibited under the dense habitation of *E. acicularis* through its nutritional competition and allelopathic activity. Thibencarb in the 1970's was popular because it effectively controlled annual weeds and *E. acicularis*. However, in some years after using the chemical, perennial weeds such as *Sagittaria pygmaea* and *Scirpus juncoides* became the typical dominant species under no competition with annual weeds and *E. acicularis*.

Since the mid 1970's the growth in Japanese rice herbicide usage has been associated with perennial weed control. This problem is considered to have arisen due to a combination of several factors, as follows:

1. weeds tend to predominate in the competition with young seedlings,
2. labor-saving progressed, and conventional tillage by ploughing in the autumn was omitted and was replaced by simpler shallow tillage using a rotary cultivator in the early spring, which favors the growth of perennial weeds

Table 1.1 Main weeds in Japanese paddy fields

Annual species	Perennial species
Grasses	**Grasses**
Echinochloa oryzicola	*Leersia oryzoides*
E. crus-galli var. *crus-galli*	*Paspalum distichum*
Leptochloa chinensis	**Sedges**
Sedges	*Cyperus serotinus*
Cyperus difformis	*Eleocharis acicularis* var. *longiseta*
C. iria	*E. kuroguwai*
Broadleaves	*Scirpus juncoides* var. *ohwianus*
Ammannia multiflora	*S. nipponicus*
Bidens frondosa	*S. planiculmis*
B. tripartite	**Broadleaves**
Eclipta prostrate	*Alisma canaliculatum*
Ludwigia epilobioides	*Sagittaria pygmaea*
Lindernia procumbens	*S. tirfolia*
Monochoria korsakowii	*Oenanthe javanica*
M. vaginalis var. *plantaginea*	**Algae**
Persicaria hydropiper	*Spirogyra arcla*
Rotala indica var. *uliginosa*	*Pithophora zelli*

(Shibayama, 1996)

Table 1.2 Change of serious weed species in Japanese paddy fields under herbicide applications

Years	Application method	Main herbicides	Noxious weed species
Before 1950's	Rotary & hand weeding		Annual and some perennial weeds
1950's	Foliar application	2,4-D, MCPA	*Echinochloa oryzicola*
1960's	Soil application	pentachlorophenol chlornitrofen nitrofen	*Eleocharis acicularis*
1970's	Foliar & soil application	thibencarb simetryn molinate	*Sagittaria pygmaea* *Scirpus juncoides* *Cyperus serotinus*
1980's	One-shot application	pyrazolate bensulfuron-methyl pyrazosulfuron -ethyl mefenacet	*Eleocharis kuroguwai* *Sagittaria trifolia*
1990's	New formulation for labor-saving		Annual broadleaves

(Shibayama, 1996)

mainly through regeneration from rhizomes,

3. herbicide use was confined to mildly acting herbicides which are highly safe for young seedlings [3].

Sequential treatment of soil incorporation or soil application herbicides and foliar and soil application herbicides was commonly practiced to achieve sufficient weeding of annual and some perennial weeds. For the first weeding 4-5 days after rice transplanting, the important products utilized were oxadiazon, butachlor and thibencarb which were effect on annual and some perennial weeds. At the second weeding, a mixture of MCPB, molinate and simetryn was most widely used product for broadleaf weeds. However, using this sequential treatment, the average number of applications in Japan was 2.2-2.4 annually but differences in rice growing conditions could mean that the actual number of applications varies considerably from region to region. In general, early post-emergence treatment has increased at the expense of pre-emergence treatments (Fig. 1.2). In order to improve systematic weed control programs which involve the frequent use of herbicides at different time, it has become necessary to develop a broad spectrum herbicide with long-lasting weed control activity which can control the weeds, including the perennial ones, and which can cope with the problem of a prolonged weed emergence period caused by mechanical planting methods [4, 5]. There was a little difference in infested acreage of annual weeds between districts in Japan (Table 1.3). On the other hand, the occurrence and growth of perennial weeds depended on these districts, e.g. *Scirpus* species were dominant in Hokkaido and Tohoku districts, but not in Kyushu and Shikoku districts [4].

The advent of "one-shot" herbicide, which was a combination product of two or three chemicals to control both annual and perennial weeds by once application, has significantly altered the paddy rice herbicide applications in Japan since the first product of pyrazolate (pyrazole herbicide, 1st generation of one-shot) was introduced

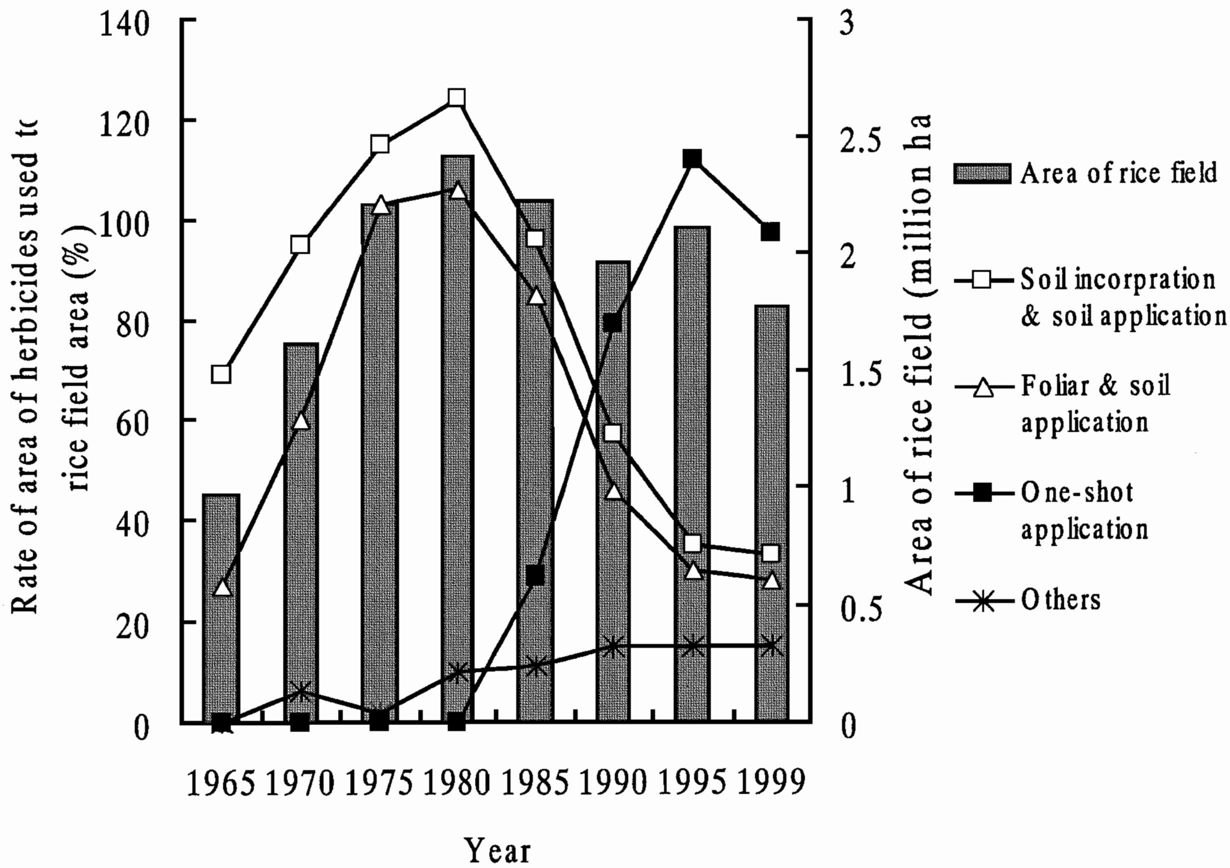


Fig. 1.2 Rice herbicide use in Japan
(From the statistics of JAPR)

Table 1.3 Infested area of paddy weeds in every regions in Japan

	Rate of area of rice field (%)								
	Hokkaido	Tohoku	Kanto	Hokuriku	Tokai	Kinki	Chugoku	Shikoku	Kyushu
Annual weeds	%	%	%	%	%	%	%	%	%
Echinochloa oryzicola	88	87	85	94	83	91	81	91	85
Monochoria vaginalis	6	29	33	40	40	39	36	53	36
Rotala indica	4	11	16	9	19	16	15	36	31
Cyperus difformis	4	14	26	11	26	28	21	36	28
others	34	32	35	34	37	48	28	43	36
Perennial weeds									
Eleocharis acicularis	41	30	30	45	42	41	33	33	24
Scirpus juncoides	84	60	43	27	23	38	32	20	14
Sagittaria pygmaea	11	22	35	43	47	55	40	55	57
Alisma canaliculatum	72	24	12	2	5	11	8	5	4
Sagittaria trifolia	11	44	22	15	9	10	7	10	5
Cyperus serotinus	4	36	28	32	16	17	18	17	15
Eleocharis kuroguwai	—	20	12	5	5	7	6	7	2
Potamogeton distinctus	9	12	11	5	19	23	21	6	10
Oenanthe javanica	2	12	11	5	19	23	21	6	10

(Miyahara, 1992)

in 1982. Especially, combination of pyrazolate and butachlor controlled paddy weeds by once by early-post transplanting application. The use of one-shot herbicides attracted attention because of their many expected advantage, including a lesser impact on the environment due to the reduced herbicide load, and a reduction in costs accompanying the decrease in the number of applications. However, there was still room for further improvement. One improvement that could be made was the development of herbicide with long-lasting activity which could inhibit weed emergence for a longer period of time. Another was the development of herbicide for which the application time was flexible. Although right treatment before emergence has been said to be a keystone for weed control by herbicides, in some situations farmers were very busy with farmwork between puddling of the herbicides [5]. Development of an effective herbicide against *Eleocharis kuroguwai* and *Sagittaria trifolia*, which persisted due to their characteristic ecology of emergence and against which conventional herbicides were ineffective, was awaited.

Agricultural Utility of Sulfonylurea Herbicides

The sulfonylurea herbicides (Fig 1.3) were discovered in the mid-1970s and immediately set a new standard for chemical weed control [6]. Their most dramatic feature is based on extremely high herbicidal potency. The herbicides provide broad-spectrum weed control at less than 100 g/ha. Such low use-rate help solve handling, application and container-disposal issues, while reducing the amount of the chemical applied to the field by a factor of 10-100 times lower than conventional herbicides [7].

The widespread use of sulfonylurea herbicides for weed control in wheat and barley began with the commercialization of chlorsulfuron by DuPont in 1981 [6]. However, this product suffered from persistence problems which due to its high activity could result in carry over problems in to subsequent crops. Since that time, second-generation products offering less persistence have been commercialized for

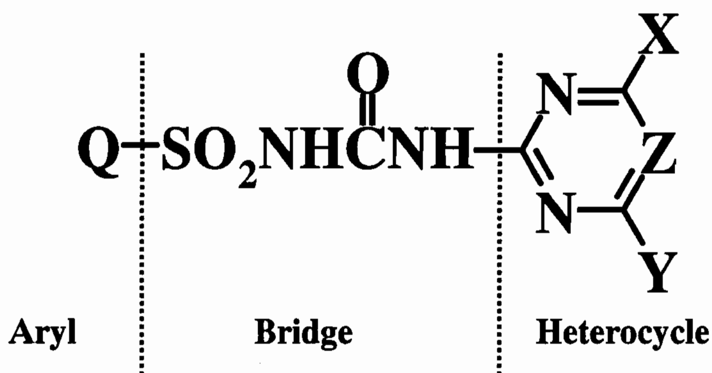


Fig. 1.3 General formula of sulfonylureas

use in all the major crop sectors (Table 1.4).

A majority of sulfonylurea herbicides have been commercialized for use in graminaceous crops. However, this broad class of chemistry has also yielded commercial products with selectivities towards broadleaf crops including soybeans, peanuts, oilseed rape and sugar beets. Sulfonylurea tolerant soybeans were brought to the market and allowed significantly higher rates of chlorimuron and thifensulfuron to be applied without risk of crop damage. Since the introduction of sulfonylureas, their sales have increased year by year. However, the market for the sulfonylurea based products on soybeans was again impacted by the introduction of Roundup Ready® soybeans, with the result that all products sold in this sector suffered a further drop in sales in 1999 (Fig.1.4) [8].

Bensulfuron-methyl (2nd generation of one-shot) was the first sulfonylurea herbicide commercialized in 1987 for use in rice [9]. During 10 years after development of bensulfuron-methyl, other 5 sulfonylureas (pyrazosulfuron-ethyl, imazosulfuron, azimsulfuron, ethoxysulfuron and cyclosulfamuron) were launched (Table. 1.5). These herbicides have highly herbicidal activity to annual and perennial broadleaf weeds and sedges including *Eleocharis kuroguwai* and *Sagittaria trifolia* at a dosage of 10-100 times lower than conventional herbicides. However, they are less active against *Echinochloa crus-galli* [10-12] and are sold as a premixture with other barnyardgrass herbicides to provide complete control of this and other *Echinochloa* species in countries such as Japan where the market demands a high degree of weed control. Over 100 one-shot rice herbicide combinations are currently available, and has been used in 1.4 million ha, 81% of rice planted area in 1999 (Fig. 1.6) [8].

Currently, labor time for weeding in paddy fields has been saved owing to the simplification of application methods, including 1) reduction of the amount of granule

Table 1.4 Agricultural utilities of sulfonylureas

Cereals (Wheat, Barley): 9 herbicides
Chlorsulfuron, Metsulfuron-methyl, Triasulfuron, Tribenuron-methyl, Thifensulfuron-methyl, Amidosulfuron, Sulfosulfuron, Flupyrsulfuron-methyl, Iodosulfuron
Maize: 5 herbicides
Nicosulfuron, Primisulfuron, Rimsulfuron, Halosulfuron, Prosulfuron
Soybean: 2 herbicides
Chlorimuron-ethyl, Oxasulfuron
Other crops (managed turf, oilseed rape, sugarbeet, non-crop): 4 herbicides
Sulfometuron-methyl, Flazasulfuron, Ethametsulfuron-methyl, Triasulfuron

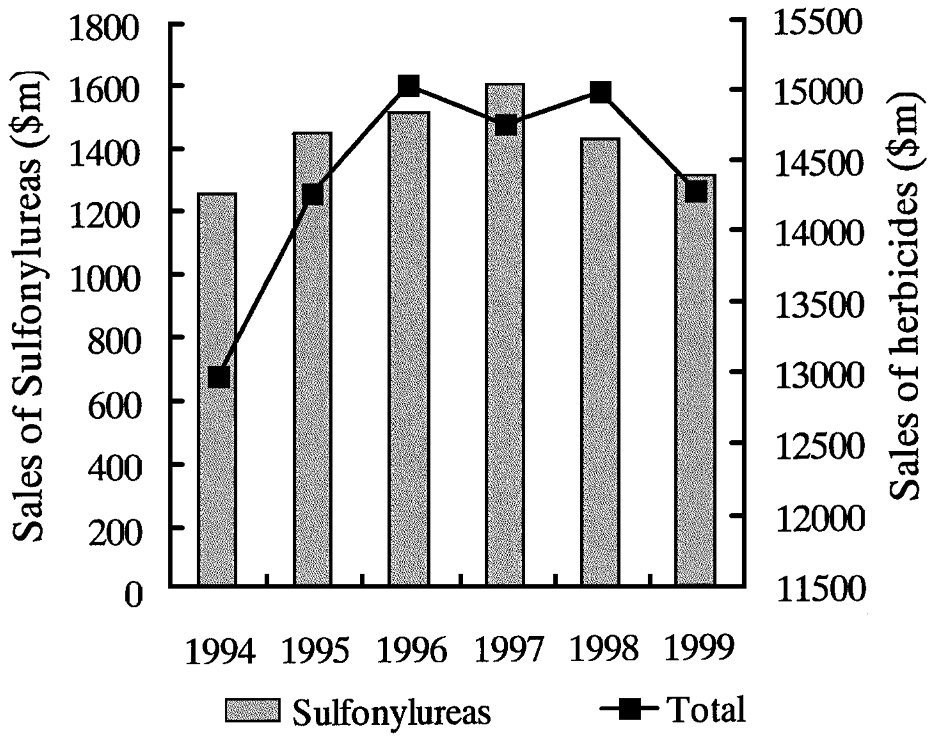
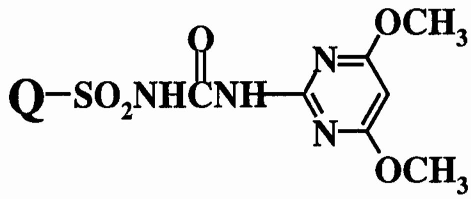
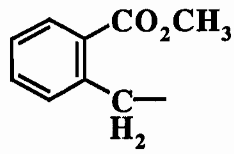
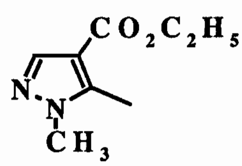
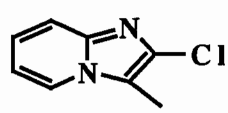
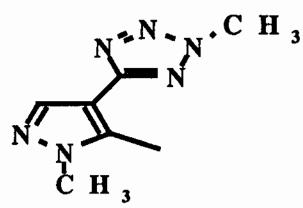
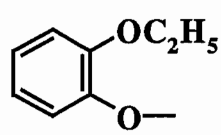
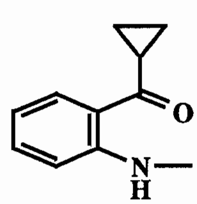


Fig. 1.4 Global market share of sulfonylurea herbicides [8]

Table 1.5 Rice sulfonylurea herbicides



Common Name	Q	Application Rate (g a.i./ha)
Bensulfuron-methyl		20-75
Pyrazosulfuron-ethyl		20-30
Imazosulfuron		75-90
Azimsulfuron		6
Ethoxysulfuron		10-30
Cyclosulfamuron		20

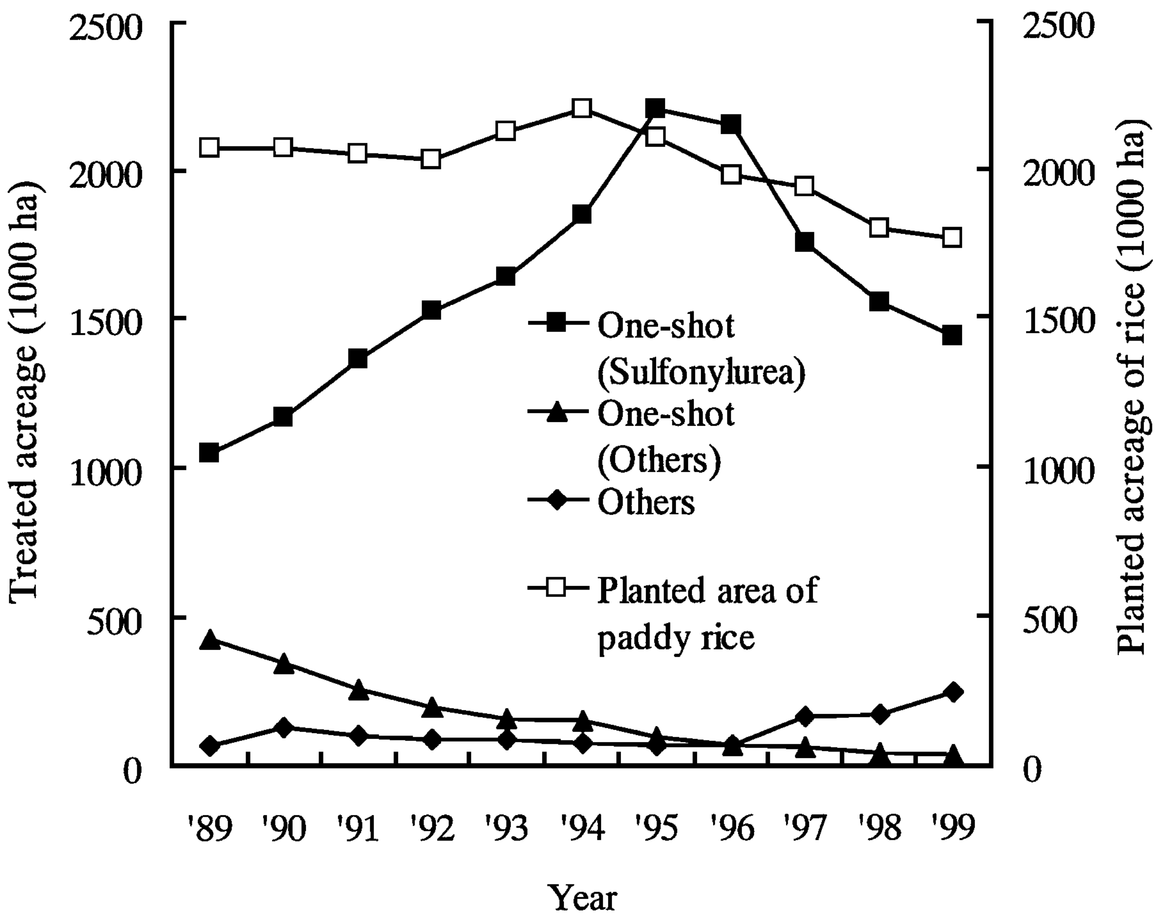


Fig. 1.5 One-shot herbicide use in Japan
(From the statistics of JAPR)

formulation applied from 30 to 10 kg/ha, and 2) development of suspension concentrate (Flowable) and throw-in type formulations (Jumbo) which can be easily applied into paddy fields from their levees, as well as reduction of the application frequency by the development and introduction of one-shot herbicides (Table 1.6).

Considering these circumstances, there is a continuous need for updating herbicide technology because of changes and developments in agricultural practices. Additionally, it is important for practical use to identify the weed control spectrum of a new herbicide in comparison with the existing herbicides. Furthermore, new herbicides possessing good crop selectivity and strong activity against new weed problems including annual and perennial weeds are requested to be developed. With the ever increasing demands made on a new herbicide and the rising costs of development, it is necessary to be able to make clear determinations on its biological activity as early as possible.

Table 1.6 Labor-saving formulations of sulfonylurea combinations

Common name	1 kg-dose granules	Suspension concentrate (Flowable)	Throw-in type formulation (Jumbo)	Total
Bensulfuron-methyl	35	26	4	65
Pyrazosulfuron-ethyl	17	4	1	22
Imazosulfuron	7	4	0	11
Azimsulfuron	0	7	0	7
Ethoxysulfuron	2	0	1	3
Cyclosulfamuron	6	0	0	6

Factors Affecting Herbicidal Activity

It is desirable for herbicides to control weeds during the season of application, but they should not do that. The behavior and selectivity of a herbicide under field conditions are the sum of a great number of factors. Many factors fall into three categories: weed and crop responses, herbicidal properties and environment (Fig. 1.6). These categories strongly interact with one another.

1. Factors of weed and crop responses

For factors of weed and crop responses affecting the herbicidal activity, the following seven factors in both weeds and crops may be considered: age, growth rate, morphology, physiology, biophysical processes (adsorption by plant cell, membrane stability), biochemical processes (herbicide inactivation, herbicide activation) and inheritance [13].

Younger plants with much meristematic tissues exhibit higher total biological activities. Therefore, the age of a plant often determines its response to a particular herbicide; young plants are less tolerant than older ones. Probably for the same reason, the growth rate of plants has pronounced effects on their reaction to some herbicides. In general, fast-growing plants are more susceptible to treatment than slow-growing plants are.

The morphology of a plant can determine whether or not it can be killed by a specific herbicide. Morphological differences are found in three places: 1. root systems, 2. location of growing points, and 3. uptake parts [13]. Annual weeds in a perennial crop can be controlled because most annual weeds have shallow root systems, whereas perennial weeds have deep, extensive root systems. Such deep root systems may escape efficacy from a herbicide that remains near the soil surface through depth-protection, whereas the shallow roots may be killed [14]. Growth inhibition of *Scirpus juncoides* var. *ohwianus* by some soil applied rice herbicides was less when the plants emerged from a deeper soil layer because distribution of their

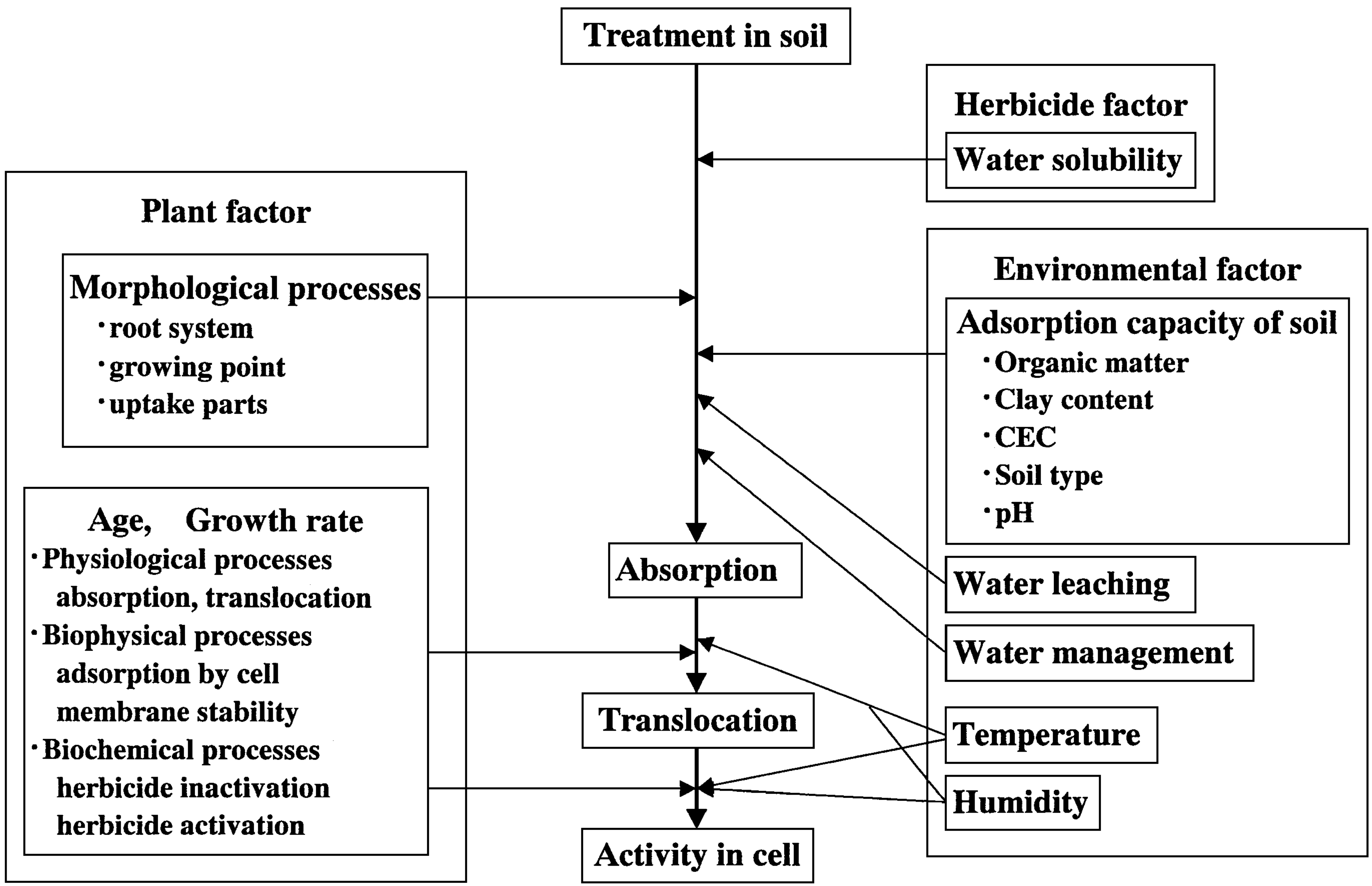


Fig. 1. 6 Scheme of probable mechanisms of factors affecting herbicidal activity

growing point was below the soil surface treated with herbicides [15]. Anderson [16] describes the differences in morphology of the emerging shoots of various broadleaf plants and grasses. Shoots parts in broadleaf weeds that are important for herbicide uptake are the hypocotyl, epicotyl and cotyledons, whereas the most important shoot parts in grasses are the coleoptile and the mesocotyl [17]. Herbicides are absorbed from the soil and flooding water by seeds, roots, shoots, rhizome, bulbs and tubers [16]. Herbicidal activity of ACN on *Sagittaria pygmaea* depended on the growth rate due to the difference in tuber size [18].

The physiology of the plant also determines the amount of herbicide taken up by the plant (absorption) and how it moves within the plant (translocation). Generally, plants that absorb and translocate the greatest amount of herbicide will be killed.

The genetic complement of a plant determines the extent to which it responds to its environment. Many of these response are morphological, physiological, biophysical or biochemical. These responses vary from genus to genus, but within a genus, plant reactions to a given herbicide tend to be similar [13]. There was locality difference in sensitivity of *Sagittaria pygmaea* to herbicides and in ecological property [19-21].

2. Factors of herbicidal properties

Herbicide uptake occurs primarily from free herbicide in soil water (22). Processes that control the concentration of herbicide in soil water are the solubility of the herbicide, the adsorptive capacity of the soil for the herbicide, and the water content of the soil. The soil components that adsorb herbicides are soil organic matter and clay. Neutral herbicides are preferentially absorbed onto the organic matter of soils (23). All sulfonylureas are weak acids having p*K*a values ranging from 3.3 to 5.2 [7]. Ionization is centered on the sulfonamide nitrogen, and at any normal soil pH there is an equilibrium mixture of the neutral and anionic forms of the molecule [7]. This ionization markedly affects the physical properties of these herbicides. In general, the soil organic carbon is highly related to soil adsorption of non-ionic pesticides [24]. Since the neutral sulfonylurea molecule is much more lipophilic than its anionic form, water solubility, soil sorption (primarily to organic matter), and soil mobility are controlled by pH [7]. Herbicidal activity of soil applied herbicide was primarily governed by its concentration in plant available soil water [25]. Constant rate of desorption of the herbicide in the soil assures the constant herbicidal concentration in the plant available soil water when used in paddy field [26].

3. Environmental factors

Control of weeds in the field by soil applied herbicides is based on the premise that the herbicides will move from the treated soil in to the weed species. Ideally a herbicide, when applied to the soil, should reach its target in the plant root zone in sufficient concentration to control target weeds for a desired period of time. Maximum herbicide performance results when the herbicide is placed in the zone of weed seed germination and seedling development. Shoot absorbed herbicides should be concentrated in the top 0 to 5 cm of soil [13]. As the weed shoot passes through the herbicide zone, uptake occurs and the seedling is killed. If the herbicide is mechanically incorporated too deeply or excessive leaching occurs, poor weed control will result. Dilution occurs if the herbicide is distributed in a larger volume of soil and water. Conventionally, many herbicides were applied at rates higher than those actually required for weed control under ideal conditions to offset losses that occur from their site of action in the plant root zone.

Highly water soluble herbicides move downward more readily with soil water [26]. However, the percent organic matter, and the type and percentage of clay particles present in a given soil affect movement of herbicides dissolved in soil water [13].

After a herbicide has dissolved in soil water, it may be adsorbed or tied up by soil particles. Factors that are most important for soil adsorption are the clay types, mineral oxides present, the percent organic matter, soil pH, and soil water content. Each soil has its own capacity to adsorb a particular herbicide. Herbicides with a low sorption index are more likely to leach in a given soil than those with a larger value. The sorption index of some herbicides is variable depending upon soil factors such as organic matter and pH. In low pH (acid) soils, the herbicide chlorimuron is less soluble and tightly bound to the organic matter [7]. In high pH (alkaline) soil, the chemical is much soluble, less tightly bound to organic matter, and subject to leaching. Solubility and the sorption index are important indicators of herbicide mobility and impact weed control effectiveness.

Leaching of herbicides can occur as rainfall or irrigation water moves down through soil. Leaching potential of various herbicides depends on factors, solubility, amount and frequency of rainfall, soil adsorption, persistence, and soil texture and structure. Water management in paddy field is very important. Good water management brings good results with the use of any herbicides. Variations in the position of roots or other organs taking up herbicide in relation to the location and availability of herbicide in soil water make the selective action of soil applied herbicide as depth protection [27]. However, this selectivity without some physiological tolerance tended to be affected by the water leaching [28].

Warm temperatures and high humidity often increases herbicidal action in relation to the following factors: 1. better absorption of the herbicides into plants, which is mainly depending on transpiring stream, 2. increase in the physicochemical reaction of the herbicides against plants cells under a high temperature, 3. change in the physiological and biochemical states of plants [27, 29].

The bioassay for factors affecting a herbicidal activity contribute to a better understanding of the field performance of herbicides which are in a development stage. The influence of diversity of factors is in most cases impossible to attribute completely when the performance of a product has to be evaluated under field conditions. A special difficulty also is the multiple interdependence among themselves of the factors aforementioned. It is, therefore, necessary to carry out tests which separate these factors under conditions which can be controlled artificially; this is only possible under laboratory conditions. There are great differences in occurrence and growth of the weeds, and rice growth from region to region in Japan due to climate, soil types, cultivation period of rice. Residual activity and safety against many varieties of rice plants needs to be carried out.

Mode of Action and Crop Selectivity Mechanism of Sulfonylureas

Rice tolerance to bensulfuron-methyl and pyrazosulfuron-ethyl also results from rapid metabolic inactivation. Under standardized conditions, rice plants metabolized these herbicides with a half-life less than 6 h while sensitive weed species metabolized them much more slowly. Further, the direct analog of bensulfuron-methyl without the bridge methylene substituent is not readily metabolized by rice plants.

Sulfonylureas were potent inhibitors of plant growth and usually exhibit growth cessation in susceptible plants very shortly after application. Sulfonylureas exhibited herbicidal activity by both soil and foliar treatments. The most common symptom in plants was general yellowing of the foliage accompanied by shortening of plant internodes followed by foliar necrosis and plant death [6]. This herbicide group may also show some secondary symptoms which include reddening of foliage, abscission of leaves, vein discoloration and terminal bud death. The symptoms were generally slow to develop in susceptible plants with death not occurring for one to two weeks after application [7, 11]. Sulfonylureas were highly mobile once taken up by plants and were translocated in both xylem and phloem [30].

Branched-chain amino acids, valine, isoleucine and leucine, reversed the inhibition of growth and cell division induced by these herbicides [31-34]. Their modes of action were studied by Ray [38]. The primary site of action is the inhibition of acetolactate synthase (ALS; EC 4. 1. 3. 18, also referred as acetohydroxyacid synthase: AHAS), an important enzyme in the pathway for biosynthesis of branched-chain amino acids (Fig. 1.7).

Although chlorsulfuron and imazapyr did not affect protein synthesis, they were associated with a decrease in DNA and RNA synthesis [35-37]. Rost [36] showed that sulfonylureas blocked the cell cycle of pea root meristem cell in G1 and G2 phase and

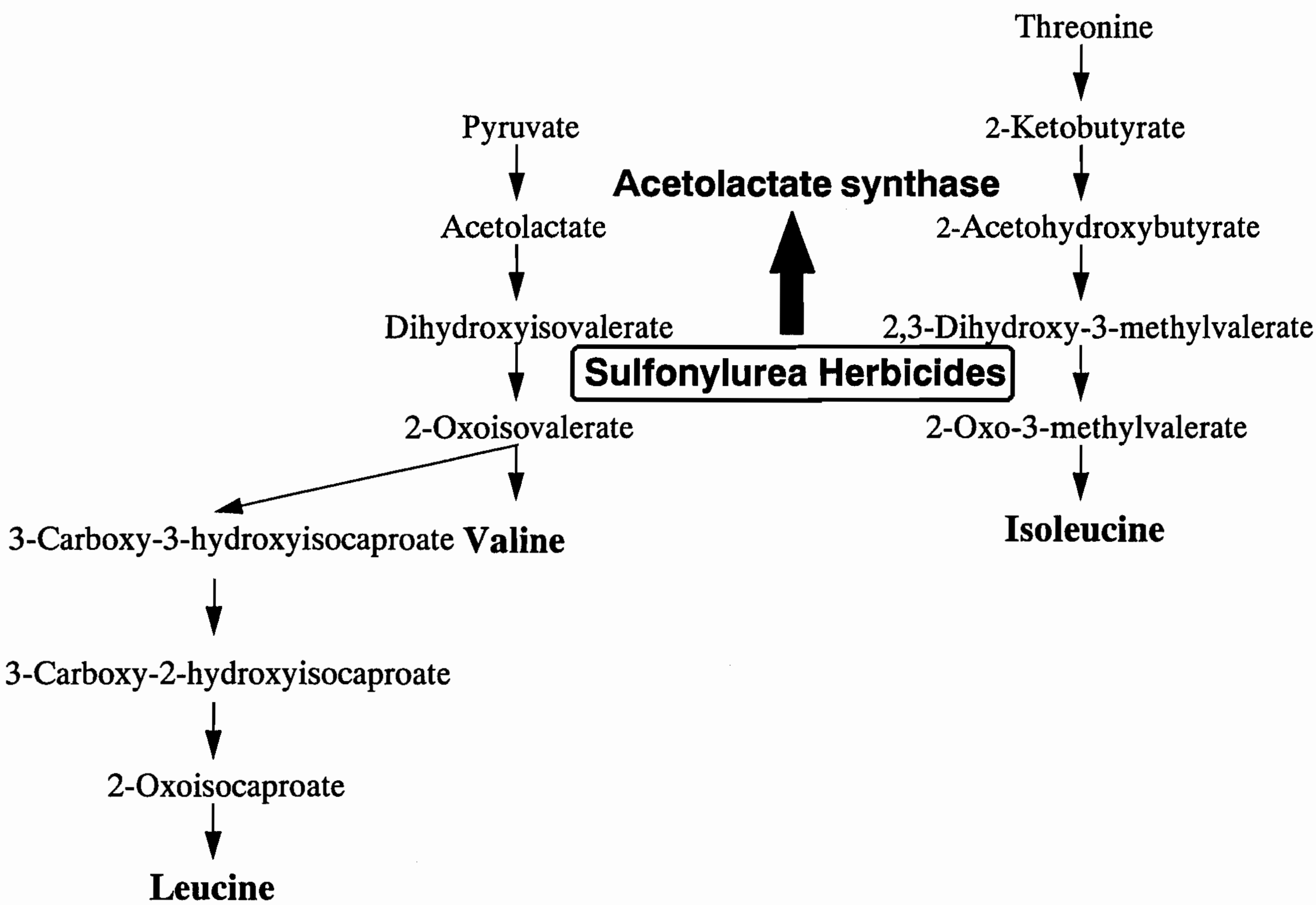


Fig. 1.7 Biosynthesis pathways for valine, leucine and isoleucine in plants

hypothesized that G2 was the primary block and G1 the secondary block. Other studies have also indicated that these chemicals indirectly affect cell division. Ray [38] demonstrated that sulfonylureas did not directly inhibit DNA synthesis by inhibition of DNA polymerase. Likewise, DNA synthesis in isolated nuclei was unaffected by herbicide treatments and supplementing sulfonylurea-treated tissue with DNA precursors, such as deoxyribonucleosides or nucleotides, did not alleviate cell division inhibition in corn roots [38].

The general hypothesis for the mode of action was that these herbicides work by depleting the branched-chain amino acid pool size and thereby perhaps causing the depleting of cell-cycle-specific proteins or nucleic acids [36, 37].

The sulfonylurea inhibited the ALS activity from plants in noncompetitive or mixed-type inhibitions with respect to pyruvate [39-41]. Close proximity on the enzyme between the herbicide, thiazole ring of thiamine pyrophosphate (TPP) and isoalloxazine ring of flavine adenine dinucleotide (FAD) was suggested [42]. In extended-time-course experiments, sulfonylurea herbicides have been shown to act as biphasic, slow-binding inhibitors [31, 40, 43] .

The extractable ALS activity in the plant leaves was based on fresh weight or total protein development [44], where the enzyme is known to be localized [45], and rapid cell division [44]. The enzyme activity from a wide variety of plant species, including both tolerant and sensitive plants, was sensitive to sulfonylurea inhibition [31].

The crop tolerance to various types of sulfonylurea was not due to difference in the sensitivity of ALS to herbicide inhibition (Table 1.7). Inherent plant tolerance to sulfonylurea herbicides is based on rapid metabolic inactivation by tolerant plants [7, 44, 46] (Fig. 1.8). Metabolic capabilities and pathways vary widely among plant species. Among these metabolic capacities are found aryl and aliphatic hydroxylation and glucose conjugation, deesterification, aryl nucleophilic displacement, oxidative *O*-demethylation, urea-bridge hydrolysis and sulfonamide bond cleavage. However, the chlorsulfuron-insensitivity of the

Table 1.7 Sulfonylurea herbicide inhibition of ALS isolated sensitive and tolerant plants [2, 13]

Plant	ALS I_{50} (nM) Bensulfuron-methyl	Pyrazosulfuron-ethyl	Chlorsulfuron	Chlorimuron-ethyl
Wheat	13.6	-	21.8*	5.1
Rice	16.3*	13.8*	24.0	5.8
Soybean	74.6	-	32.4	7.7*
Wild mustard	12.6	-	10.9	3.1
Morning-glory	97.9	-	24.3	6.8
Pea	63.8	-	20.9	6.0
Barnyardgrass	21.8	16.2	24.3	3.6

ALS I_{50} shows the concentration causing 50% inhibition of ALS activity. Asterisked values indicate tolerance of that plant species to the corresponding sulfonylurea herbicide.

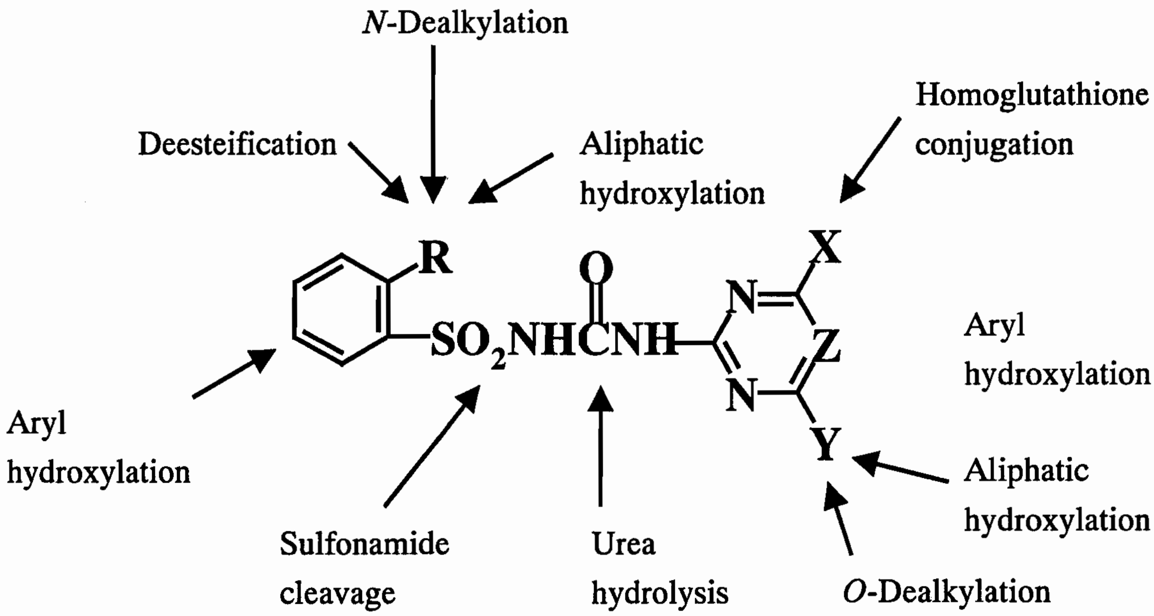


Fig. 1.8 Metabolism of sulfonylureas in plants

legume plants at early growth stage after germination depended on the contents of both ALS and acetoin forming enzyme which was involved in regulating the supply of amino acids for storage protein synthesis [47]. The general hypothesis [48] for the mode of action was that ALS inhibitors work by depleting the branched-chain amino acid pool size and there by perhaps causing the depleting of cell-cycle-specific protein or nucleic acids. On the other hand, the build up of 2-ketobutyrate have been proposed to be quite toxic to cell (Fig. 1.9).

Although the biochemical site of these ALS inhibitors has been identified, the connection among inhibition of ALS, the rapid cessation of plant cell division, changes in amino acid profiles and general growth inhibition remains an area of active research.

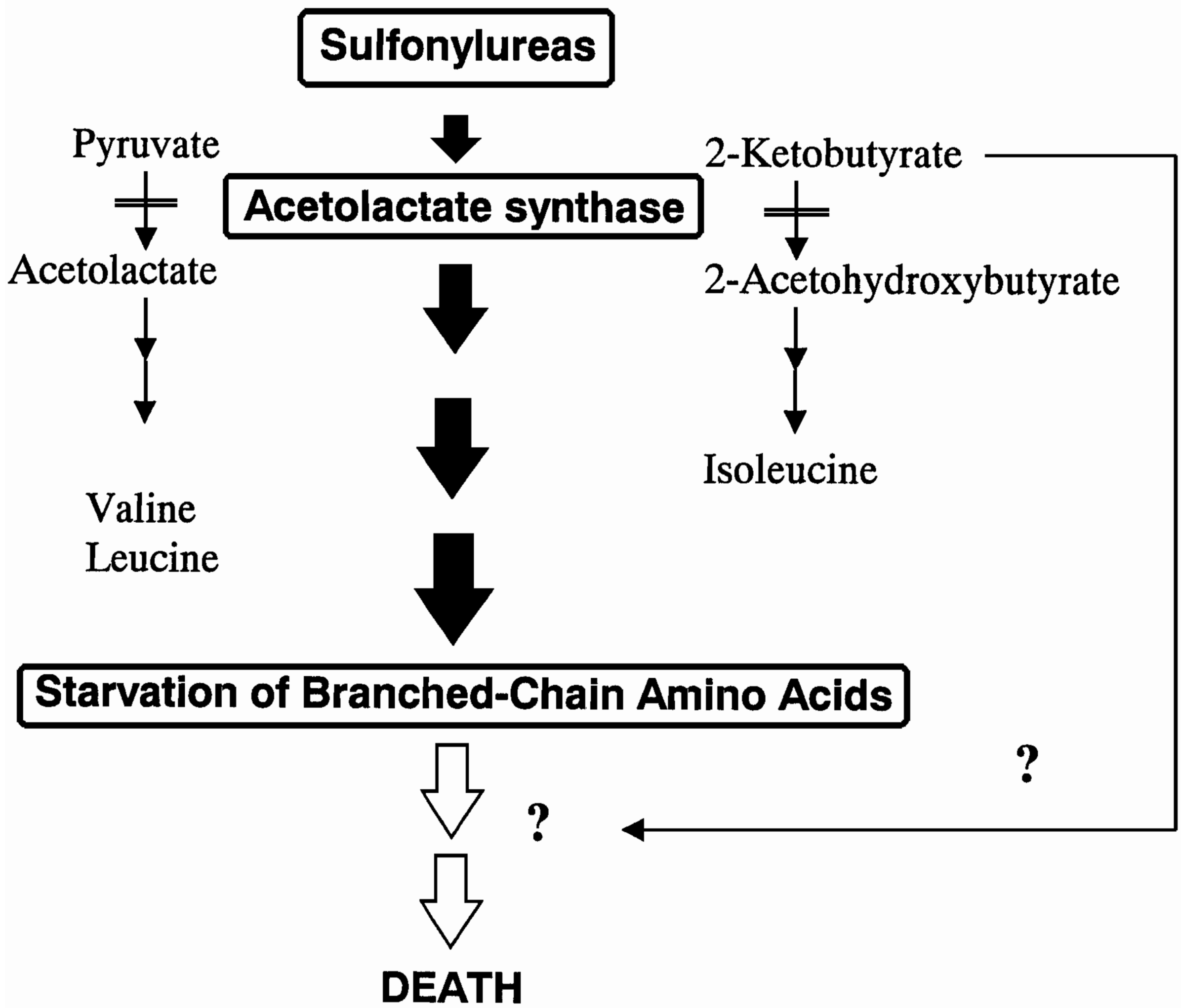


Fig. 1.9 Scheme of the physiological responses with respect to the mode of sulfonylurea action

Imazosulfuron

Herbicide investigation aimed at development of high performance herbicide for paddy rice was started in 1984. Introduction of bicyclic heterocycles into the aryl moiety of sulfonylurea was carried out with utilizing much knowledge of heterocyclic compounds, benoxazol and fentiazon [49]. As a result, imazosulfuron, (1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea), was discovered [49, 50] and developed for annual and perennial broadleaf and sedge weeds in paddy field in 1985.

In the aryl moiety, substitution at 2-position was very important for the herbicidal activity and crop selectivity. Imazosulfuron has a chlorine at 2-position on the imidazo[1,2-*a*]pyridine instead of methoxycarbonyl, ethoxy, ethoxycarbonyl, and cyclopropylcarbonyl at the same poison in the aryl moiety of the other sulfonylureas (Table 1.5), and is the only compound with halogen substituent in the moiety.

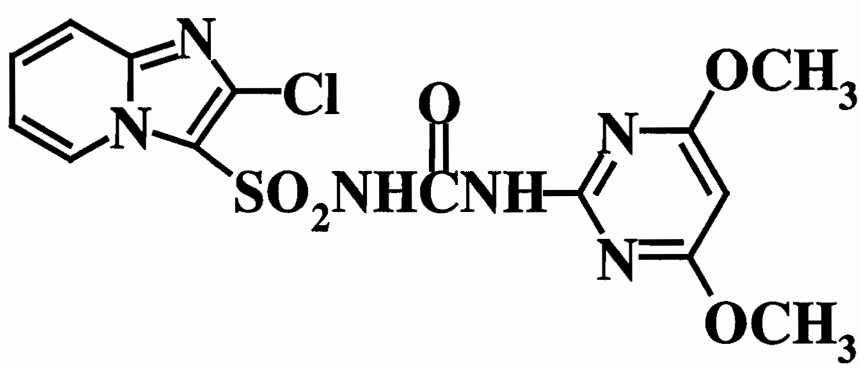
Imazosulfuron was monodemethylated in excised leaves of rice plants and *C. serotinus*, and the difference in metabolic rate of imazosulfuron between these plants is concerned in the selectivity [51].

Adsorption of imazosulfuron was dependent on soil types, and adsorption equilibrium reached in 2 hr. However, its Freundlich adsorption coefficient values were not correlated with the content of soil organic carbon but were highly correlated with soil cation exchange capacity and soil pH [52]. Low affinity to organic carbon was due to the dissociation of imazosulfuron, having a p*K*a 4.0 [49], in the soils of pH 4.7 to 6.0 [52] as observed with other acidic compounds [53]. Imazosulfuron was hardly desorbed from the soil, and its mobility decreased with time irrespective of soil type [52]. Imazosulfuron in the paddy soils under aerobic conditions gradually disappeared with time by the hydrolysis of the sulfonylurea bond to give ADPM (2-amino-4,6-dimethoxypyrimidine) and IPSN (2-chloroimidazo[1,2-*a*] pyridine-3-sulfonamide), and finally mineralized to carbon dioxide [54]. On

Table 1.8 Chemical and physical properties of the herbicide imazosulfuron [49, 50]

Common name : imazosulfuron
Code name : TH-913
Chemical name :
1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-di-methoxypyrimidin-2-yl) urea (IUPAC)

Structural formula :



Molecular weight : 412.83
Appearance : crystalline white powder
Specific gravity : 4.574 (25℃)
Melting point : 183-184℃ (dec.)
Vapor pressure : 3.4×10^{-10} mmHg (25℃)
Solubility (mg/*l*, 25℃) :

water	5 (pH5.1), 67 (pH6.1), 308 (pH7.0)
acetonitrile	2,500
ethyl acetate	2,200
dichloromethane	12,800
xylene	400

Partition coefficient (log P_{OW}) : 0.05 (pH7)
Dissociation constant (pKa) : 4.0

the other hand, imazosulfuron under anaerobic conditions was degraded by soil microorganisms, followed by conversion to soil organic matters. Therefore, imazosulfuron is not accumulated at least in the flooded soils [54]. Thus, imazosulfuron is considered to be safe, and has a minimal impact on the environment.

Objective

In this paper, imazosulfuron was studied for biological activity, factors affecting biological activity, selectivity and mode of action in order to predict the behavior of the herbicide under the field conditions for the practical use.

In chapter 2 and 4, herbicidal activity on paddy weeds, phytotoxicity on rice plants and factors affecting these activities were investigated. First, herbicidal activity on 12-weed species, residual activity of imazosulfuron was examined. The bioassays for factors affecting a herbicidal activity and phytotoxic property to rice plants contribute to a better understanding of the field performance of herbicides. Moreover, there are great differences in occurrence and growth of weeds and rice plants from region to region in Japan due to climate, soil types and cultivation period of rice. The influence of diversity of factors, however, is in most cases impossible to attribute completely when the performance of a herbicide has to be evaluated under field conditions. It is, therefore, necessary to carry out tests which separate these factors under conditions which can be controlled artificially; this is only possible under greenhouse conditions. Environmental and plant factors such as exposure period, tuber size, flooding water depth, temperature, water leaching and planting depth were carried out for the herbicidal activity, and transplanting time, flooding water depth, water leaching, transplanting depth and temperature for the phytotoxicity to rice plants. Additionally, movement of imazosulfuron in paddy field soil was examined for predict of the herbicide activity and the phytotoxicity to rice plants under field conditions, and for establishment of its practical use. Bioassay method was carried out using SCPJO and CYPDI.

In chapter 3, selectivity and site of absorption were carried out. Selectivity of a herbicide is in many cases a combined effect which is based on pure physiological selectivity on the one hand and selectivity by depth protection on the other. Therefore, the site of absorption (root or shoot) influences the selectivity of a compound. Selectivity between rice plants and paddy weeds were investigated using SCPJO, ECHOR, SAGPY and CYPSE for the paddy weeds. Three application methods were used to estimate the absorption site of imazosulfuron in CYPSE and in rice plants. They are soil incorporation for root absorption, flooding water application for shoot absorption, soil application for both root and shoot absorption.

In chapter 5, mode of action was carried out. From both scientific and practical viewpoints, it is greatly important to elucidate the mode of action of herbicide. These tests enable compound with reduced environmental risk to be selected for further study. Sulfonylurea inhibits ALS, an important enzyme in the pathway for biosynthesis branched-chain amino acids. Alleviative effects of branched-chain amino acids on the growth inhibition by imazosulfuron were investigated using rice plants. Additionally, the inhibitory effects on ALS prepared from 6 plants (ECHOR, SAGPY, CYPSE, TRIAE, ORYSA, and PISSA) were examined. Investigation for inhibition properties of imazosulfuron on ALS was carried out using PISSA ALS.

In chapter 6, physiological responses of CYPSE to imazosulfuron in combination with valine, isoleucine and leucine were investigated. As a result, imazosulfuron was thought to directly inhibit ALS from the plants, however, the connection among inhibition of ALS, the rapid cessation of plant division and general growth inhibition remains an area of active research. First, alleviation effect of branched-chain amino acids on the inhibitory growth of CYPSE induced by imazosulfuron was investigated. Additionally, effects on protein, RNA and DNA synthesis, soluble protein levels and free amino acids levels were examined.

CHAPTER 2

HERBICIDAL ACTIVITY AND FACTORS AFFECTING HERBICIDAL ACTIVITY AGAINST PADDY WEEDS

Performance of rice paddy herbicides is usually affected by environmental factors such as temperature, water leaching, soil properties and water management as well as conditions of weeds and crops. The bioassays for factors affecting a herbicidal activity contribute to a better understanding of the field performance of herbicides. The influence of diversity of factors, however, is in most cases impossible to attribute completely when the performance of a herbicide has to be evaluated under field conditions. It is, therefore, necessary to carry out tests which separate these factors under conditions which can be controlled artificially; this is only possible under greenhouse conditions. Environmental and plant factors such as exposure period, tuber size, flooding water depth, temperature, water leaching and planting depth was carried out. Additionally, movement of imazosulfuron in paddy soil was examined for predict of the herbicide activity and the phytotoxicity to rice plants under field conditions, and for establishment of its practical use. Therefore, this chapter describes the herbicidal activity and factors affecting herbicidal activity of imazosulfuron.

MATERIALS AND METHODS

The following experiments were conducted in a greenhouse. Shugakuin clay loam soil (organic matter content = 5.11 %) was placed in 200 cm^2 plastic Wagner pots. Unless cited otherwise, ten seeds of ECHOR and SCPJO were planted in the flooded soil at a depth of 0.5 cm. Seedlings were thinned to 5 plants per pot just before herbicide application. Tubers of SAGPY, SAGTR, CYPSE, SCPPL, ELOKU and bulbs of PTMDI were planted in the flooded soil at 1 cm to 2 cm depth. A part of the runner segments of OENJA was put into the flooded soil. Seedlings of these plants were thinned to 3 to 5 plants just before application. Seeds of MOOVA, LIDPY, CYPDI and ROTIN mixed with soil were broadcasted on the flooded soil surface and covered with a thin layer of soil. These weed seeds and tubers were harvested in Shugakuin in Kyoto. Flooding water was kept constant at 5 cm depth without water leaching during the tests. The plants were harvested and the dry weights of the above-ground parts were measured. Sampling time in each experiment is given in the following. Each experiment had three replications.

Herbicidal spectrum and residual activity

For assay of herbicidal spectrum, immediately after planting or when weeds reached the indicated leaf stage, imazosulfuron was applied at 90 g a.i./ha as pre-emergence or post-emergence treatments. The plants were harvested 21 or 28 days after applications. Weed control efficacy against MOOVA, LIDPY and ROTIN was evaluated visually in comparison with the untreated plants (0 %, no effect- 100 %, complete kill).

For assay of residual activity, imazosulfuron was applied at 75 g a.i./ha. Immediately after the application, 14, 28 or 42 days after the application, seeds of SCPJO and tubers of SAGPY and CYPSE were planted as mentioned above. After 21 days from the planting, weed control efficacy was evaluated visually as mentioned before.

Movement in paddy soil

A test was conducted using a plastic case 10 cm×10 cm×28 cm in size filled to within 5 cm from the top with puddled paddy soil (Shugakuin sandy clay loam, organic matter = 4.78%). After water was added to a depth of 4 cm above the soil, imazosulfuron was applied at 150 g a.i./ha. Flooding water was drawn down at the rate of 1.5 cm/day one day after this application until fully drained. The soil column was removed from the case and divided into 1 cm cross-sections, then each section was placed in a plastic pot 6 cm in diameter and mixed thoroughly. Seeds of CYPDI and SCPJO were planted in flooded soil in pots. Weed control efficacy was evaluated visually in comparison with the untreated plants 28 days after applications.

Effect of exposure period

Seeds of SCPJO, ECHOR and CYPSE were planted in the flooded soil. When they had reached the 1.2, 1.0, 1.0-leaf stage, respectively, a suspended solution, prepared by dissolving technical imazosulfuron in acetone and bringing the solution to volume in 0.1 % (v/v) polyoxyethylene (20) sorbitan monolaurate (TWEEN 20®) was applied at 90 g a.i./ha. Plants were removed from the treated flooded soil in pots and rinsed in water 1, 2, 3, 7 or 14 days after the application, then transplanted to a non-treated flooded soil in pots, and harvested 28 days after applications.

Effect of tuber size

Small tubers (20-40 mg fresh weight/pre-sprouting tuber), medium ones (60-80 mg), large ones (exceeding 100 mg) of SAGPY were planted in the flooded soil. At sprouting stage or when they reached the 1.0-leaf stage, a suspended solution as described above was applied at 3 or 90 g a.i./ha. The plants were harvested 35 days after the application.

Effect of flooding water depth

Seeds of SCPJO and tubers of SAGPY and CYPSE were planted as above. When they reached the 1.6, 3.4, 3.3-leaf stage, respectively, imazosulfuron was applied at 75 g a.i./ha and flooding water depth was maintained at 1 cm or 5 cm during the tests. The plants were harvested 28 days after the application.

Effect of temperature after application and water leaching

Seeds of SCPJO were planted in the flooded soil. Immediately after planting or when they had reached the 1.5-leaf stage, imazosulfuron was applied at 75 g a.i./ha. Thereafter plants were kept in growth chambers controlled at 22℃/15℃, 26℃/19℃ or 33℃/26℃ (day/night) temperature regime, and tests were done under water leaching or non-leaching conditions. Water leaching was regulated by an electric pump and conducted at the rate of 3 cm/day for 3 days beginning the day after application. Flooding water was maintained at a depth of 5 cm during the tests, and the plants were harvested 28 days after the application.

Effects of planting depth

Seeds of SCPJO were planted in flooded soil in Wagner pots at 0.5 cm or 2 cm depth. Immediately after planting or when plants had reached the 2.0-leaf stage, imazosulfuron was applied at 50 or 100 g a.i./ha. The plants were harvested 28 days after application.

Tubers of SAGPY were planted in flooded soil at 2 cm or 5 cm depth. Immediately after planting, imazosulfuron was applied at 50, 75 or 100 g a.i./ha, and plants were harvested 35 days after application.

Chemical

Granular formulation of 0.25 or 0.3 % imazosulfuron was used (Table 2.1).

Table 2.1 Description of imazosulfuron 0.25% granules

Imazosulfuron	Active ingredient	0.25%
Carboxymethy cellulose	Binder	1.5%
Montmorillonite	Binder	15%
Sodium lignosulfonate	Dispersing agent	2.0%
Polyoxydiethylenenonylphenylether	Wettable agent	0.5%
Clay		Balance

RESULTS

Herbicidal spectrum

Herbicidal spectrum of imazosulfuron was extensive (Fig. 2.1). Especially the growth of annual broadleaf weeds (MOOVA, LIDPY and ROTIN at cotyledon stage) and perennial weeds (SAGPY, SAGTR, OENJA, PTMDI, SCPJO and CYPSE) was strongly suppressed to the degree above 95% as compared with the untreated control. With post-emergence applications, imazosulfuron inhibited elongation of their shoots and killed them gradually. The symptoms first appeared in the meristematic tissue where growth ceased soon after application, followed by slight chlorosis and then necrosis. The dry weight of ECHOR, SCPPL and ELOKU were reduced more than 85% compared with that of control, however, they were alive and remained green while being stunted. With pre-emergence applications, some of emerged ECHOR elongated to coleoptile or 1-leaf stage and then all stunted about 10 days after applications.

Residual activity

Emergence of SCPJO, SAGPY and CYPSE was almost suppressed for 42 days to the degree above 78% (Fig. 2.2). After sprouting in treated paddy soil, CYPSE ceased growing shortly thereafter irrespective of planting time after applications of imazosulfuron. On planting 28 days following application, some of SAGPY and SCPJO began to emerge and elongated to coleoptile or 1 leaf-stage but all remained stunted without further growth.

Movement in paddy soil

Relative movement of imazosulfuron in paddy soil was assessed by a bioassay method. CYPDI and SCPJO seeded in the uppermost segment (0 - 1

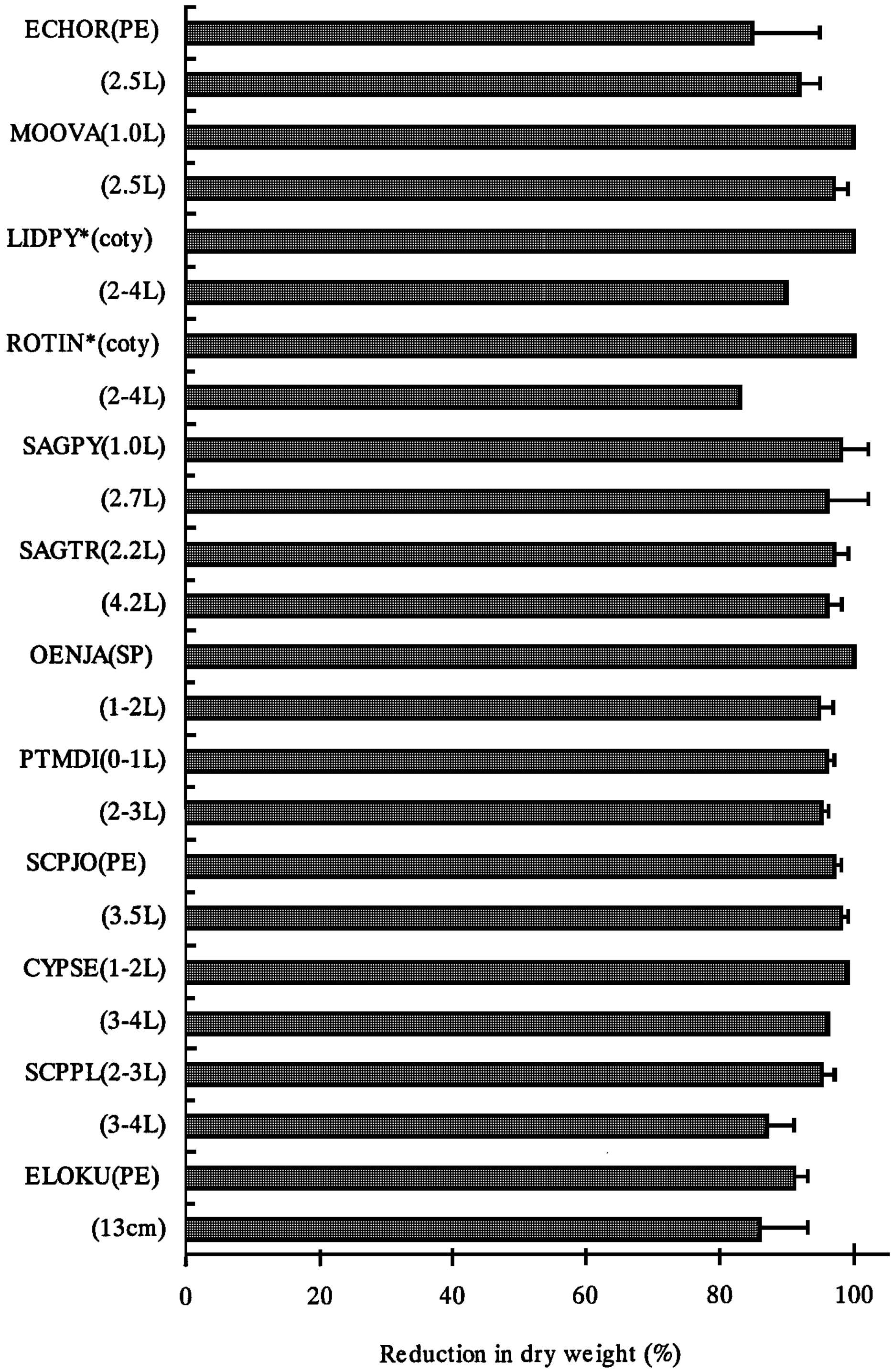


Fig. 2.1 Herbicidal effect of imazosulfuron at 90 g a.i./ha on 12 paddy field weeds
Horizontal bars represent standard deviations. Numbers and letters in parentheses show leaf stages or plant heights at applications. Asterisk indicates visual evaluation (0%, no effect; 100%, complete kill). Plants were harvested 21 or 28 days after applications.
PE, pre-emergence stage; coty, cotyledon stage; L, leaf stage; sp, sprouting stage

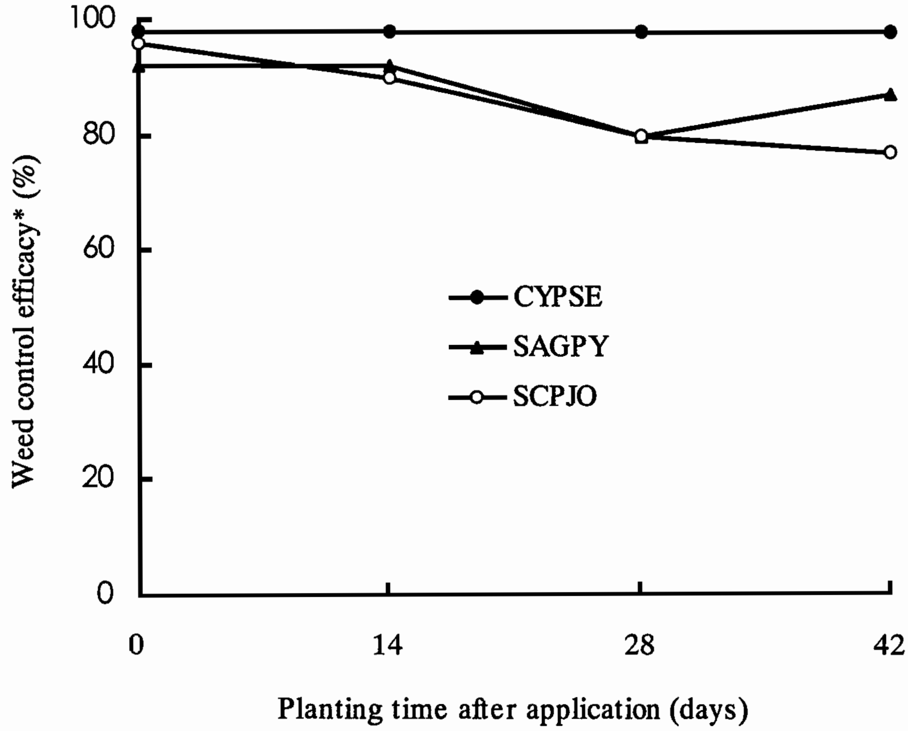


Fig. 2.2 Residual activity of imazosulfuron at 75 g a.i./ha against CYPSE, SAGPY and SCPJO.
*Visual evaluation of weed control was made (0%, no effect; 100%, complete kill) 28 days after applications.

cm) of the soil layer suffered the greatest growth inhibition, those seeded in the second segment (1 - 2 cm) the next, those in the third segment (2 - 3 cm) the least and those in the balance of the soil none (Fig. 2.3). Weed control efficacy of imazosulfuron on CYPDI was greater than that on SCPJO.

Effect of exposure period

Strong reduction of over 90% in dry weight was observed in CYPSE, SCPJO and ECHOR treated with imazosulfuron for 1 day or more, 7 days or more and 14 days or more, respectively (Fig. 2.4). This reduction was roughly proportional to the exposure period of between 1 and 7 days with different sensitivities. The sensitivities of ECHOR, SCPJO and CYPSE to imazosulfuron increased in this order.

Effect of tuber size

By applications of 3 g a.i./ha at sprouting stage, the growth of SAGPY suffered significantly less reduction in dry weight with increasing tuber size (Fig. 2.5). Similar reduction was shown with application at the 1-leaf stage. The difference in growth inhibition between small and large tubers at the sprouting stage was greater than that at the 1-leaf stage. With 90 g a.i./ha at the sprouting stage, however, the growth of all SAGPY was suppressed completely irrespective of tuber size.

Effect of flooding water depth

The growth of the three weeds was suppressed more than 80% relative to untreated controls, when the flooding water depth was either 1 cm or 5 cm (Fig. 2.6). There was a slight sensitivity difference in herbicidal activity among them but no remarkable difference between these two depth.

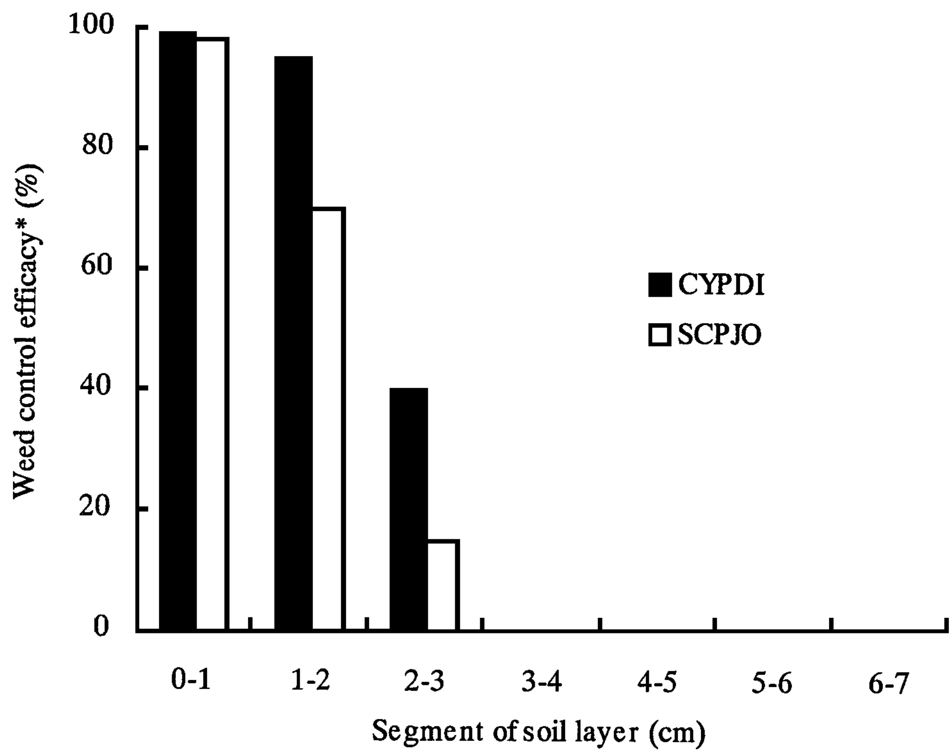


Fig. 2.3 Movement of imazosulfuron in a sandy clay loam soil
*Visual evaluation of weed control efficacy (0%, no effect; 100%, complete kill) was made.

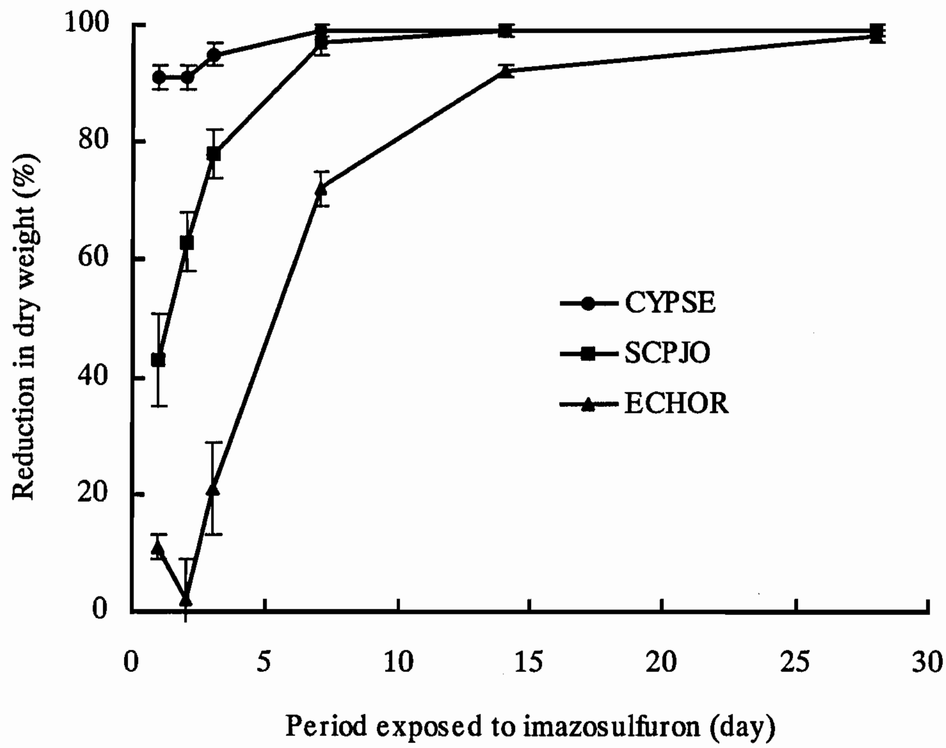


Fig. 2.4 Effects of the period exposed to imazosulfuron on its herbicidal activity against CYPSE, SCPJO and ECHOR.
Vertical bars represent standard deviations. Dry weights of untreated control without being transplanted are as follows.
CYPSE, 13.71±1.31 g/pot; SCPJO, 1.77±0.11 g/pot; ECHOR, 8.24±0.84 g/pot

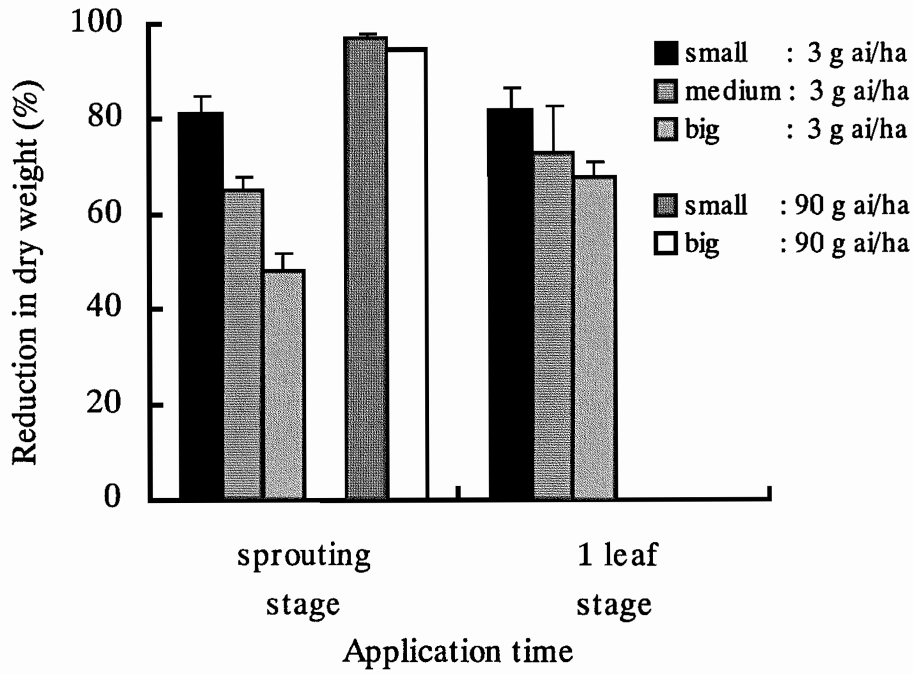


Fig. 2.5 Effects of the tuber size of SAGPY on herbicidal activity of imazosulfuron
Vertical bars represent standard deviations.
Small tuber, 20-40 mg (fresh weight/pre-sprouting tuber)
medium tuber, 60-80 mg (fresh weight/pre-sprouting tuber)
big tuber, >100 mg (fresh weight/pre-sprouting tuber)
Dry weights of untreated control (sprouting stage, 1-leaf stage) are as follows (mean $\pm$ SD).
small, (0.36 $\pm$ 0.02, 1.06 $\pm$ 0.09 g/pot); medium, (0.49 $\pm$ 0.03, 1.08 $\pm$ 0.11 g/pot); big (0.52 $\pm$ 0.02, 1.29 $\pm$ 0.11 g/pot)

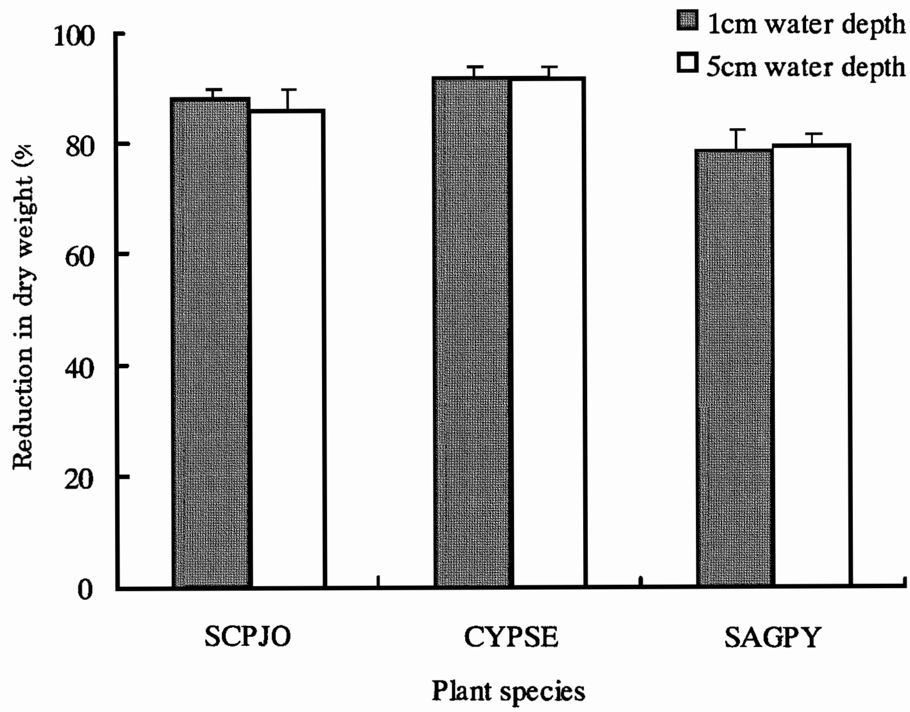


Fig. 2.6 Effects of the flooding water depth on herbicidal activity of imazosulfuron against SCPJO, CYPSE and SAGPY Vertical bars represent standard deviations. Dry weights of untreated control plants (1 cm depth, 5 cm depth) are as follows (mean ± SD).

SCPJO, (0.12 ± 0.03 g/pot, 0.12 ± 0.01 g/pot); CYPSE, (2.23 ± 0.10 g/pot, 2.35 ± 0.31 g/pot); SAGPY, (0.31 ± 0.04 g/pot, 0.27 ± 0.05 g/pot)

Effect of temperature after applications and water leaching

Temperature after application had no appreciable influence on the reduction in dry weight of SCPJO (Fig. 2.7). With pre-emergence application of imazosulfuron, the growth inhibition of this plant under non-leaching conditions was significantly higher than that under water leaching conditions. Similar inhibition was shown by 1.5-leaf stage applications; however, the difference in growth inhibition by application at this stage between water leaching and non-leaching was less than that with pre-emergence application. Under non-leaching conditions there was no remarkable difference in the growth inhibition irrespective of the difference in application time, while under water leaching conditions the growth reduction by 1.5-leaf stage application was significantly larger than that by pre-emergence.

Effect of planting depth

With 2-leaf stage applications of 50 g a.i./ha and 100 g a.i./ha, no significant difference was found in the growth inhibition of SCPJO planted at either 0.5 cm or 2 cm depth (Fig. 2.8). Pre-emergence application caused significantly larger growth reduction of the plants seeded at 0.5 cm than those planted at 2 cm. In particular, the difference in reduction of dry weight by application of 50 g a.i./ha between the plants seeded at 0.5 and 2 cm was greater than that by application of 100 g a.i./ha.

With pre-emergence application, SAGPY planted at 2 cm depth suffered significantly more growth reduction than that planted at 5 cm irrespective of dosage (Fig. 2.9).

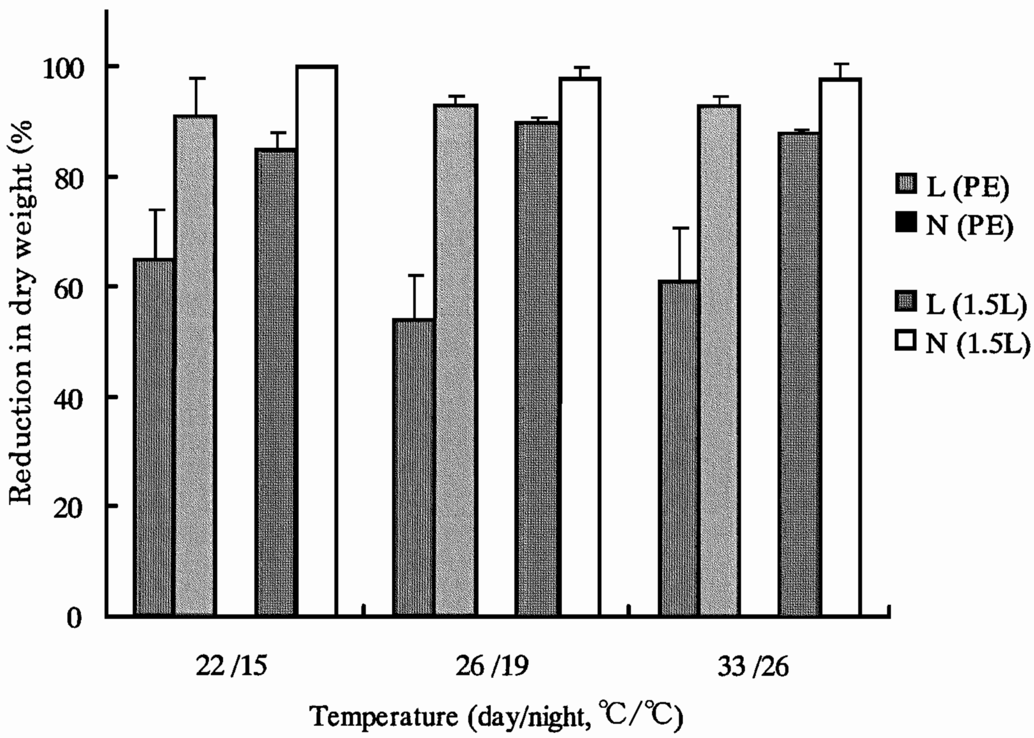


Fig. 2.7 Effects of temperature after application and water leaching on herbicidal activity of imazosulfuron at 75 g a.i./ha against SCPJO

Vertical bars represent standard deviations.

L, water leaching conditions; N, non-leaching conditions;

PE, pre-emergence stage application; 1.5L, 1.5-leaf stage application

Dry weights of untreated control plants (22/15, 26/19, 33/26) are as follows (mean ± SD).

L(PE), (0.03 ± 0.00, 0.05 ± 0.00, 0.12 ± 0.01 g/pot); N(PE), (0.03 ± 0.00, 0.14 ± 0.02, 0.17 ± 0.02 g/pot); L(1.5L), (0.08 ± 0.01, 0.18 ± 0.02, 0.25 ± 0.02 g/pot); N(1.5L), (0.14 ± 0.00, 0.41 ± 0.06, 0.44 ± 0.01 g/pot)

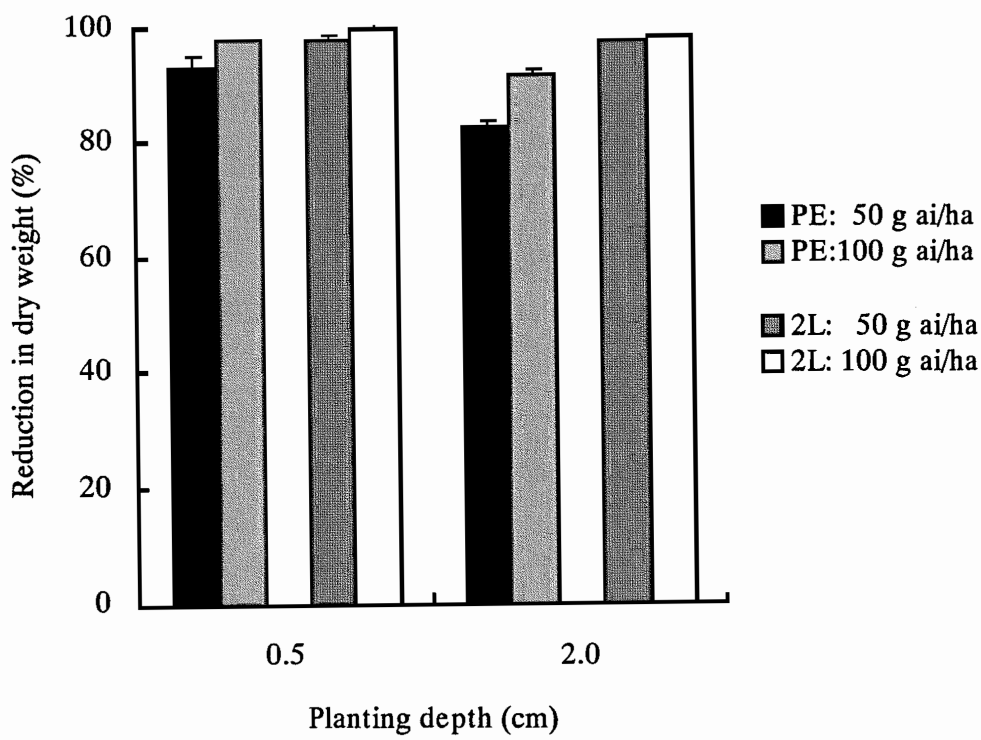


Fig. 2.8 Effect of planting depth of seeds of SCPJO on herbicidal activity of imazosulfuron
Vertical bars represent standard deviations. Dry weights of untreated control plants (0.5 cm depth, 2.0 cm depth) are as follows (mean ± SD).
PE, (0.21 ± 0.02, 0.15 ± 0.02 g/pot); 2L, (0.28 ± 0.03, 0.55 ± 0.07 g/pot)

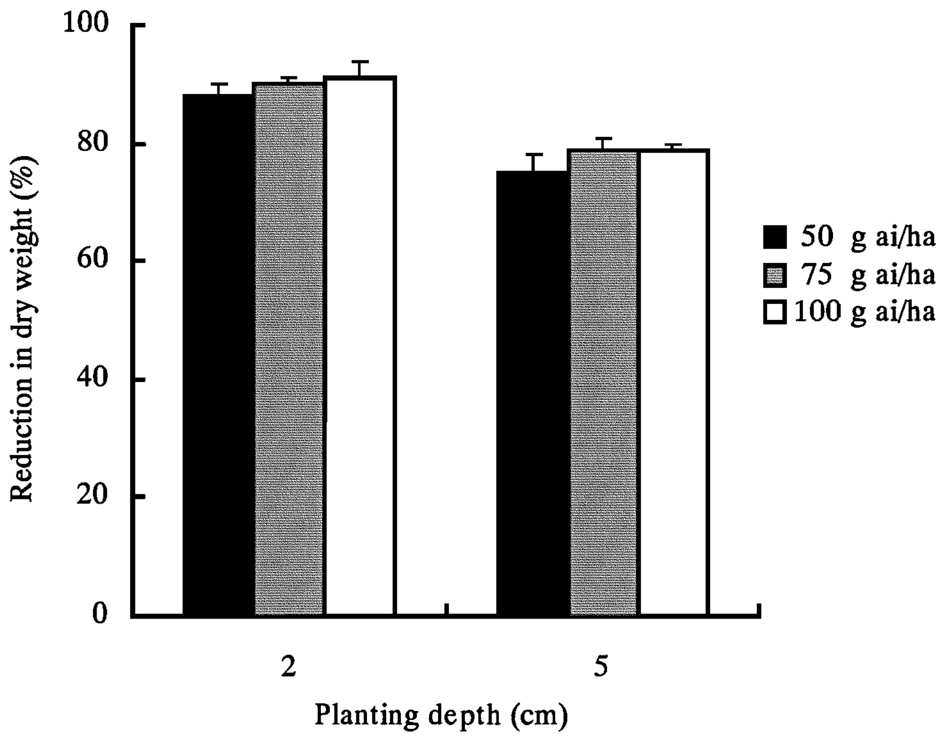


Fig. 2.9 Effect of planting depth of tubers of SAGPY on herbicidal activity of imazosulfuron

Vertical bars represent standard deviations. Dry weights of untreated control plants are as follows (mean ± SD).

2 cm depth, 0.53 ± 0.06 g/pot; 5 cm depth, 0.48 ± 0.05 g/pot

DISCUSSION

Imazosulfuron had a high herbicidal activity on most paddy weeds (Fig. 2.1). Most susceptible weeds eventually died after severe stuntedness while some moderately susceptible weeds were stunted remained green. In a field, however, these weeds were severely suppressed in competitive with rice plants. A period to eventual plant death after application seems to depend on the sensitivity of the weeds to imazosulfuron.

Imazosulfuron suppressed emergence and growth following emergence of these weeds for approximately 42 days (Fig. 2.2). These seem to be a different efficacy due to the difference of their sensitivity to the herbicides. Sensitivity of CYPSE to the herbicide may be the highest, SAGPY the next, SCPJO the lowest. Inhibition of emergence of these weeds gradually decreased with delaying planting time, however, their growth ceased 1 leaf or the former stage. Similar inhibition was reported in Chinese cabbage treated with bensulfuron-methyl [11]. Thus growth inhibition ceased 1 leaf or the former stage of these weeds may be due to the different sensitivity between emergence and seedling stage to imazosulfuron. Efficacy of imazosulfuron on *Echinochloa oryzicola* is higher than that of bensulfuron-methyl and pyrazosulfuron-ethyl, but not in practical use, and need a combination with a grass killer herbicide. There are differences in sensitivity of weeds on these herbicides. However, at a practical dosage, there is a little difference in herbicidal efficacy between them. Therefore, these herbicides are used as main products of a combination with grass and sedge killer herbicides, but not as a single application.

Movement of imazosulfuron in paddy soil under water leaching was limited to within 1 cm of the soil but enough to inhibit the emergence of SCPJO. Difference in efficacy on between SCPJO and CYPDI may be due to difference in sensitivity to the herbicide, which may depend on biochemical processes in the plants.

Under non-leaching condition, the vertical movement of imazosulfuron from the soil surface downwards might be limited to less than 1 cm of soil, which is enough to inhibit the growth of SCPJO. Movement downwards in the soil depended on the adsorption to the soil and water solubility of herbicides [53, 56, 57]. The soil components that adsorb herbicides are soil organic matter and clay [23]. Ionization is centered on the sulfonamide nitrogen, and at any normal soil pH there is an equilibrium mixture of the neutral and anionic forms of the molecule [7]. This ionization markedly affects the physical properties of these herbicides [7]. Since the neutral sulfonylurea molecule is much more lipophilic than its anionic form, water solubility, soil sorption (primarily to organic matter), and soil mobility are controlled by pH [7]. Adsorption of imazosulfuron was dependent on soil types, and adsorption equilibrium reached in 2 hr. However, its Freundlich adsorption coefficient values were not correlated with the content of soil organic carbon but were highly correlated with soil cation exchange capacity and soil pH [52]. In general, the soil organic carbon is highly related to soil adsorption of non-ionic pesticides [24]. Low affinity to organic carbon was due to the dissociation of imazosulfuron, having a p*K*a 4.0 [49], in the soils of pH 4.7 to 6.0 [52] as observed with other acidic compounds [53]. Imazosulfuron was hardly desorbed from the soil, and its mobility decreased with time irrespective of soil types. Imazosulfuron is a weak acid [49] as other sulfonylurea [6, 7], and its water solubility depends on pH (Table 1.3). Movement of bensulfuron-methyl in paddy soil was limited to less than 2 cm of the soil and similar to that of butachlor. Soil adsorption of bensulfuron-methyl, which has a p*K*a 5.2, was not correlated with soil pH between 5 and 6. Since the soil pH was generally close to its p*K*a, the influence of pH on soil binding was not apparent. Pyrazosulfuron-ethyl (p*K*a 3.7) adsorption to soil was also significantly related to soil pH [57]. Less movement of imazosulfuron in the soil might be due to the high adsorption to the soil and the low desorption from the soil depending on the lower p*K*a. Further studies are needed to investigate the effects of the ratio of neutral/acid form and change of soil pH on movement of imazosulfuron in the soil and soil water.

The period for eventual plant death after application of imazosulfuron seems to depend on the weed sensitivity to it. Plant response to a herbicide is affected by plant age, growth rate, morphological processes, physiological processes, biophysical processes, biochemical processes and inheritance [13]. This difference in sensitivity might also play a part in the herbicide efficacy with extreme decrease in the treatment period or dosage applied to the soil surface.

There was locality difference in sensitivity of SAGPY to herbicides and in ecological property [19-21]. The herbicidal activity of ACN on the plants depended on the growth rate due to the difference in tuber size [18]. When the level of imazosulfuron on the soil surface is greatly decreased the tuber's size of SAGPY might be a factor in efficacy at sprouting stage. However, this does not seem to be a factor affecting the growth reduction with a 90 g a.i./ha dosage of imazosulfuron for a practical use.

It is remarkable that the difference in flooding water depth did not significantly affect the herbicidal activity of imazosulfuron.

The difference in temperature after application does not seem to affect the herbicidal activity of imazosulfuron.

In a non-leaching condition, imazosulfuron had great efficacy on SCPJO with both pre- and post-emergence application but greater efficacy on ECHOR, which is a moderately susceptible weed to the herbicide, with post-emergence application than pre-emergence one. The decrease in initial grow of SCPJO with pre-emergence application in a leaching condition (Fig. 2.7) might be due to the lesser amount of imazosulfuron on the soil surface induced by the leaching and the difference in efficacy between pre- and post- emergence application. Herbicides are absorbed from the soil and flooding water by seeds, roots, shoots, rhizome, bulbs and tubers [16]. Compound is absorbed from seed with pre-emergence application, whereas from roots with post-emergence application. Therefore, the decrease might partly depend on the difference in uptake part.

Watanabe and Shibayama [15] reported that the growth inhibition of SCPJO by some herbicides was less when the plants emerged from a deeper soil layer than under standard conditions because their mesocotyl did not reach to the soil surface. This indicated that distribution of their growing point below the soil surface affected herbicide absorption. Therefore, inhibition of the growth of SCPJO and SAGPY with pre-emergence application of this herbicide could be influenced by the planting depth due to differences in the length of mesocotyl or basal shoot and distribution of the growing point in these plants. Shoots parts in broadleaf weeds that are important for herbicide uptake are the hypocotyl, epicotyl and cotyledons, whereas the most important shoot parts in grasses are the coleoptile and the mesocotyl [17]. As already indicated, amount of imazosulfuron moved downwards between 1cm and 2 cm in the soil by leaching was not enough to completely suppress SCPJO. Thus, without leaching amount of imazosulfuron being enough to completely inhibit the growth of the plants planted in a 2 cm depth do not suggest moving downwards.

Under non-leaching condition, the vertical movement of imazosulfuron from the soil surface downwards might be limited to less than 1 cm of soil. The post-emergence application of imazosulfuron, however, seemed to have stable activity on SCPJO which was planted at a depth of 2 cm because of the absorption of the herbicide from shoots.

SUMMARY

Imazosulfuron controlled 12 paddy field weeds including annual and perennial broadleaf and sedge weeds on pre- and post-emergence applications of 90 g a.i./ha. Application of 75 g a.i./ha suppressed emergence and growth afterwards of SCPJO, SAGPY and CYPSE for 42 days.

Reduction of more than 90% in dry weight was observed in CYPSE, SCPJO and ECHOR treated with 90 g a.i./ha of imazosulfuron for 1 day or more, 7 days or more and 14 days or more, respectively.

Bioassay method showed that movement in paddy soil, where the water leaching was conducted at the rate of 1.5 cm/day from the 4 cm flooding water depth following application of 150 g a.i./ha, was limited to within 2 cm of the soil layer under the soil surface.

Application of 90 g a.i./ha at the sprouting stage of SAGPY suppressed the growth in dry weight irrespective of tuber size. However, at 3 g a.i./ha growth of this plant sustained less reduction with increasing tuber size. Differences in flooding water depth and temperature had no appreciable influence on the herbicidal activity.

Growth inhibition of SCPJO by pre-emergence or 1.5-leaf stage applications under non-leaching conditions was significantly higher than that under water leaching conditions. Under non-leaching condition, there was no remarkable difference in this inhibition between pre-emergence and 1.5-leaf stage applications, while under water leaching conditions the growth reduction with the latter application was significantly larger. Growth inhibition of SCPJO and SAGPY planted at a depth 0.5 cm and 2 cm respectively, was significantly higher than planted deeper with the pre-emergence applications. With application at the 2-leaf stage of SCPJO, however, the difference in planting depth did not significantly affect the herbicidal activity.

CHAPTER 3

SELECTIVITY AND ABSORPTION SITE

Selectivity of a herbicide is in many cases a combined effect which is based on pure physiological selectivity on the one hand and selectivity by depth protection on the other. Therefore, the site of absorption (root or shoot) influences the selectivity of a compound. Selectivity between rice plants and paddy weeds were investigated using SCPJO, ECHOR, SAGPY and CYPSE for the paddy weeds. Three application methods were used to estimate the absorption site of imazosulfuron in CYPSE and in rice plants. They are soil incorporation for root absorption, flooding water application for shoot absorption, soil application for both root and shoot absorption.

MATERIALS AND METHODS

The following experiments were conducted in a greenhouse. The Shugakuin clay loam soil (Organic matter content = 5.11%) in a 200 cm^2 plastic Wagner pot was used. Flooding water was kept constant at a 5 cm depth during the tests. Unless cited otherwise, the rice plants (*Oryza sativa* cv. Nipponbare, ORYSA, harvested in Shugakuin) at 2-leaf stage were transplanted into the flooded soil in the pots.

Selectivity between Rice Plants and Paddy Weeds

The seeds of SCPJO and ECHOR were planted in the flooded soil in 0.5 cm depth. The tubers of CYPSE and SAGPY were planted in the flooded soil at a 1 to a 2 cm depth. When SCPJO, ECHOR, CYPSE and SAGPY had reached the

1.1, 1.0, 0.9, 1.2-leaf stage, respectively, a suspended solution, prepared by dissolving the solution to volume in 0.1% (v/v) polyoxyethylene (20) sorbitan monolaurate (TWEEN 20®) was applied at 1, 3, 10, 30, 100 or 300 g a.i./ha.

Rice plants were transplanted into the flooded soil in the pots at a 2 cm depth at the rate of 2 plants/hill, 2 hills/pot. Zero day or 7 days after transplanting, a suspended solution of imazosulfuron was applied at 30, 100, 300, 1000 or 3000 g a.i./ha.

The plants were harvested and the dry weights of the above-ground parts were measured to calculate I_{50} (dosage of 50% reduction in dry weight) 28 days following applications. I_{50} values were calculated by a least-squares method. The results were expressed as the mean of 3 replications.

Absorption Site in CYPSE and in Rice Plants

With soil incorporation, a suspended solution of imazosulfuron as described in section 1 was applied and then incorporated in a root zone 3 cm thickness just before planting, and 0.5 cm thickness activated carbon layer was placed above it. With flooding application, the activated carbon layer was placed above the soil surface after planting, and the solution was applied to flooding water. With soil application, the solution was applied to flooding water following planting without the activated carbon layer. Sprouting stage, 0.9 or 2.0-leaf stage of CYPSE were planted at 2 plants/pot in the soil in pots at a 2 cm depth, while rice seedlings were transplanted at the rate of 2 plants/hill, 2 hills/pot. Applications at a dosage ranging from 1 to 100 g a.i./ha for CYPSE and those ranging from 30 to 1000g a.i./ha for rice plants were done just after planting. Plant height of rice plants was measured every 7 days, and shoot dry weight of both plants was done 21 days following applications. The results were expressed as the mean of 3 replications.

RESULTS

Selectivity between Rice Plants and Paddy Weeds

Growth of paddy weeds was completely suppressed at the dosage over 10 g a.i./ha of imazosulfuron (Fig. 3.1). At the dosage of less than 3 g a.i./ha, ECHOR, SCPJO, SAGPY and CYPSE were only stunted with thick greening and progressive necrosis as secondary symptoms. On the other hand, rice plants suffered only sight reduction in the dry weight at 100 g a.i./ha, and there was no remarkable difference in growth inhibition between the rice plants applied at 0 and 7 days after transplanting at the dosage of less than 300 g a.i./ha. At the dosage over 1000 g a.i./ha, however, rice plants were seriously injured and unrecoverable, and the growth inhibition, when applied 0 day following transplanting, was larger than when applied 7 days thereafter.

The I_{50} value of CYPSE, SAGPY, SCPJO and ECHOR was 1050, 2218, 605, 487 times susceptible to imazosulfuron, respectively, as compared to rice plants applied 7 days after transplanting (Table 3.1).

Absorption Site in CYPSE and in Rice Plants

CYPSE with soil application suffered the greatest growth inhibition, that with soil incorporation application the next, that with flooding water application the least (Fig. 3.2). The plants were eventually killed at the dosage over 10 g a.i./ha with soil application and at 100 g a.i./ha with soil incorporation application. Growth inhibition was reduced more with delaying application timing of imazosulfuron, and especially the inhibition with flooding water application decreased the most (Table 3.2). On the other hand, the growth inhibition of rice plants with soil application was similar to that with soil incorporation application, and both inhibitions were higher than that with flooding water application. Plant height with flooding water application

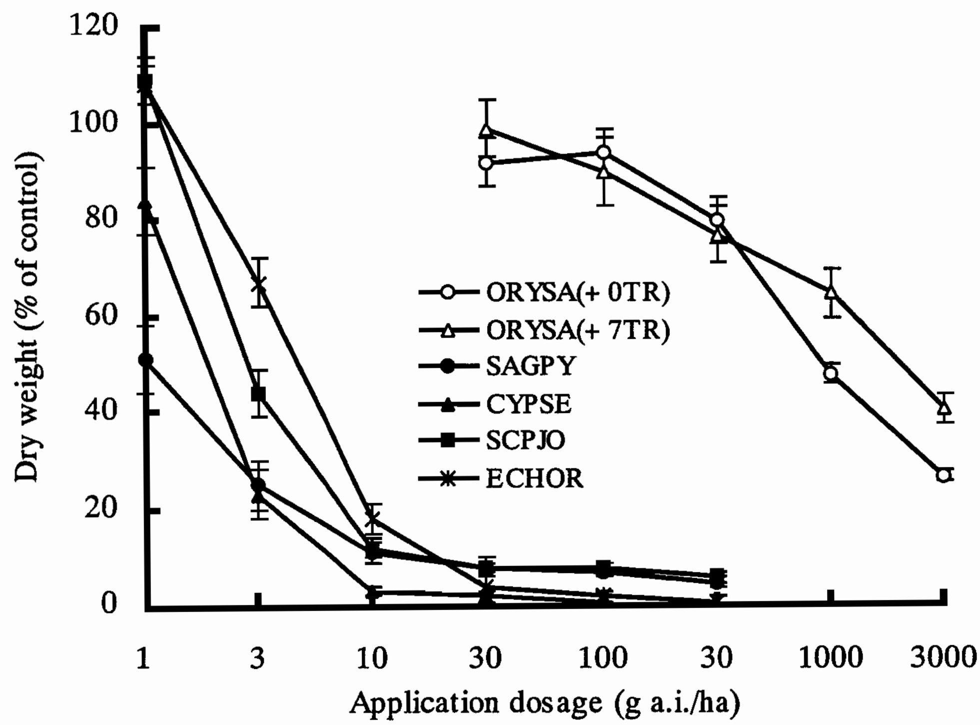


Fig. 3.1 Effect of imazosulfuron on the growth of paddy field weeds and rice plants

Vertical bars represent standard deviations.

+0 TR, treatment 0 day after transplanting; +7 TR, treatment 7 days after transplanting

The dry weight of untreated control plants is as follows:

ORYSA (+0 TR), 3.09 ± 0.21; ORYSA (+7 TR), 6.21 ± 0.27 g/hill; SAGPY, 1.06 ± 0.13; CYPSE, 4.48 ± 0.43; SCPJO, 0.12 ± 0.01; ECHOR, 2.08 ± 0.23 g/pot

Table 3.1 Difference in sensitivity among paddy field weeds and rice plants to imazosulfuron

	I_{50} (g a.i./ha)	Selectivity index	
		ORYSA (+0)	ORYSA (+7)
CYPSE	1.9	638	1050
SAGPY	0.9	1347	2218
SCPJO	3.3	367	605
ECHOR	4.1	296	487
ORYSA (+0)	1212.1	1	-
ORYSA (+7)	1995.9	-	1

I_{50}, dosage of 50% reduction in dry weight
Selectivity index was calculated by I_{50} of rice plants applied 0 day [ORYSA (+0)] or 7 days [ORYSA (+7)] after transplanting by that of weeds.

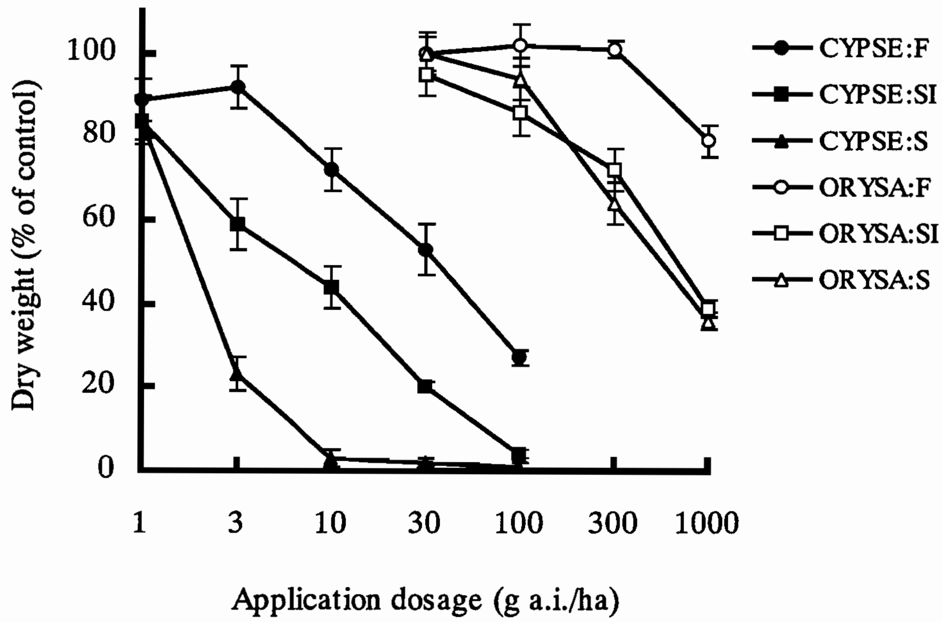


Fig. 3.2 Absorption site of imazosulfuron in 0.9 leaf-stage of CYPSE and 2 leaf-stage of ORYSA
Vertical bars represent standard deviations.
F, flooding water application; SI, soil incorporation application; S, soil application
The dry weight of untreated control plants (flooding water application, soil incorporation application, and soil application) is as follows:
CYPSE, 2.99 ± 0.23; 2.66 ± 0.27; 4.48 ± 0.43 g/pot; ORYSA, 3.30±0.10; 3.33±0.10; 3.25±0.26 g/hill

Table 3.2 Effect of application time and application method on the herbicidal activity of imazosulfuron on CYPSE

Application method*	Application timing	I_{50}**
Flooding water	sprouting stage	20
	0.9-leaf stage	38
	2-leaf stage	115
Soil incorporation	sprouting stage	8
	0.9-leaf stage	13
	2-leaf stage	23
Soil	sprouting stage	1
	0.9-leaf stage	2
	2-leaf stage	8

*Flooding water application: After planting, the activated carbon layer in 0.5 cm thickness was placed above the soil surface and the solution was applied to flooding water.
Soil incorporation: A suspended solution was applied and incorporated in soil 3 cm thickness just before planting above which activated carbon layer in 0.5 cm was placed.
Soil application: The solution was applied to flooding water after planting without the activated carbon layer.
**, Dosage (g a.i./ha) of 50% reduction in dry weight

at most excess dosage as 1000 g a.i./ha was recovered larger than that with soil incorporation application and soil application in increasing time following application (data not shown).

DISCUSSION

According to the I_{50} values, it was shown that paddy field weeds were approximately from 500 to 2200 times susceptible to imazosulfuron as compared to rice plants applied 7 days after transplanting. These results suggest that imazosulfuron has sufficient safety to rice plants at the dosage of 90 g a.i./ha for practical use.

Selectivity index, calculated by GR50 of rice plants by that of paddy weeds, of bensulfuron-methyl between rice plants and *S. juncoides* and pyrazosulfuron-ethyl between rice plants and *C. serotinus* were approximately 33 times, and 300 times, respectively. Comparing the selectivity indexes, imazosulfuron has an excellent rice herbicide with the most appropriate balance between herbicidal activity and crop selectivity.

Bensulfuron-methyl, introduced a benzyl moiety to the aryl moiety of sulfonylurea, showed good selectivity [11]. However, injury to rice plants by bensulfuron-methyl was enhanced by high temperature, resulting decrease of use dosage in South-West regions in Japan, or mixture with safeners to reduce the injury to rice plants [58]. Pyrazosulfuron-ethyl also needs in the warmer regions in Japan because of the same reason [57].

Rice sulfonylureas have the same structure in bridge and heterocycle moiety. Crop selectivity between rice plants and paddy weeds depends on the difference in monodemethylation of the pyrimidine moiety [12, 51, 59]. The direct analog of bensulfuron-methyl without the bridge methylene substituent was not readily metabolized by rice plants [7]. In the aryl moiety, moreover, substitution on 2-position is very important for the crop selectivity, even though this substitution is far from the site of monodemethylation. Imazosulfuron has a chlorine at 2-position on the imidazopyridine instead of methoxycarbonyl, ethoxy, ethoxycarbonyl and cyclopropylcarbonyl at the same position in the aryl moiety of other sulfonylureas. Therefore, the introduction of halogen, chlorine, to the 2-position enhance the safety to rice plants as well as that of the fused heterocycle moiety.

According to these results, in CYPSE imazosulfuron seems to be mostly absorbed by root and partly by shoot, while in rice plants by both root and basal leaf sheaths. The growth inhibition seems to be reduced more with delaying application timing of imazosulfuron, especially in upper part of the plant.

SUMMARY

Selectivity between paddy weeds and rice plants on imazosulfuron was investigated. I_{50} value of *Cyperus serotinus* (CYPSE), *Sagittaria pygmaea* (SAGPY), *Scirpus juncoides* var. *ohwianus* (SCPJO) and *Echinochloa oryzicola* (ECHOR) was approximately from 500 to 2200 times lower than that of rice plants treated 7 days after transplanting. This result suggests that imazosulfuron has sufficient safety to rice plants (*Oryza sativa* cv. Nipponbare, ORYSA) at a dosage of 90 g a.i./ha for practical use.

Absorption site of imazosulfuron in CYPSE and rice plants was estimated by using three application methods. CYPSE treated with soil application of the herbicide suffered the most growth suppression, that with soil incorporation application the next, that with flooding water application the least. On the other hand, the growth inhibition of rice plants with soil application was similar to that with soil incorporation application, and both inhibitions were higher than that with flooding water application.

In CYPSE imazosulfuron seems to be mostly absorbed by root and partly by shoot, while in rice plants by both root and basal leaf sheaths.

CHAPTER 4

PHYTOTOXIC PROPERTY TO RICE PLANTS

The bioassays for factors affecting phytotoxic property to rice plants contribute to a better understanding of the field performance of herbicides. Moreover, there are great differences in growth of rice plants from region to region in Japan due to climate, soil types and cultivation period of rice. It is, therefore, necessary to carry out tests which separate these factors under greenhouse conditions. Environmental and plant factors such as transplanting time, flooding water depth, water leaching, transplanting depth and temperature for the phytotoxicity to rice plants were carried out.

MATERIALS AND METHODS

The following experiments were conducted in a greenhouse. The lowest daily temperature was 18℃ and the highest was from 28 to 33℃. Clay loam soil (organic matter content = 5.11%, pH 6.7) was placed in a 200 cm^2 plastic Wagner pot. Unless otherwise stated, rice plants (ORYSA) at the 2-leaf stage were transplanted into the flooded soil in the pots. Flooding water was maintained at a 5 cm depth during the tests without water leaching.

Effect of transplanting time

Rice plants were transplanted into flooded soil in the pots at a depth of 2 cm at the rate of 3 plants/hill, 1 hill/pot. Imazosulfuron at 100 g a.i./ha or 150 g a.i./ha was applied to the flooding water 1, 5, 9 or 13 days thereafter. Dry weight of the shoots was measured 35 days after the application, and the results are expressed as the mean of 5 replicates.

Effect of flooding water depth

Rice plants were transplanted into flooded soil in the pots at a 2 cm depth at the rate of 3 plants/hill, 2 hills/pot. Imazosulfuron at 75 g a.i./ha or 150 g a.i./ha was applied 7 days thereafter. Flooding water was maintained at a 1 cm or 5 cm depth after the application, and dry weight of shoots was measured 28 days later. The results are expressed as the mean of 3 replicates.

Effect of Water Leaching

Rice plants were transplanted into flooded soil in the pots at a 2 cm depth at the rate of 3 plants/hill, 2 hills/pot. Imazosulfuron at 100 g a.i./ha or 150 g a.i./ha was applied 7 days thereafter. Water leaching at the rate of 3 cm/day was regulated by an electric pump, and continued for 3 days after the applications. Flooding water was maintained at a 5 cm depth during the tests. Shoot dry weight was measured 35 days after applications, and the results are expressed as the mean of 5 replicates.

MATERIALS AND METHODS

Effect of Transplanting Depth

For a first assay of transplanting depth, rice plants were transplanted into flooded soil in the pots at a -0.5 cm, a 1 cm or a 3 cm depth at the rate of 3 plants/hill, 2 hills/pot. Imazosulfuron at 75 g a.i./ha or 150 g a.i./ha was applied 5 days thereafter. Shoot dry weight was measured 35 days after applications, and the results are expressed as the mean of 5 replicates.

For a second assay, rice plants were transplanted into flooded soil in the pots at a 0 cm or 1 cm depth at the rate of 2 plants/hill, 2 hills/pot. With those transplanted at the 0 cm depth, basal leaf sheaths were exposed above the water and the root tips were in the soil, while the root tips of those transplanted at a 1 cm depth were exposed above the water and the basal leaf sheaths were in the soil. Zero days after transplanting, a suspended solution of imazosulfuron at 90 g a.i./ha or of pretilachlor at 600 g a.i./ha was applied. This solution was prepared by dissolving technical imazosulfuron or pretilachlor in acetone and bringing the solution to volume in 0.1% (v/v) polyoxyethylene (20) sorbitan monolaurate (TWEEN 20®). Dry weight of the shoots was measured 28 days after applications, and the results are expressed as the mean of 3 replicates.

Effect of Water Leaching and Transplanting Depth

Rice plants were transplanted into flooded soil in the pots at a 1 cm or a 3 cm depth at the rate of 3 plants/hill, 2 hills/pot. Imazosulfuron at 100 g a.i./ha or 150 g a.i./ha was applied 4 days thereafter. Water leaching at the rate of 2 cm/day was regulated by an electric pump, and continued for 3 days after the applications. Flooding water was maintained at a 5 cm depth during the tests. Shoot dry weight was measured 28 days after applications, and the results are expressed as the mean of 3 replicates.

RESULTS

Effect of transplanting time

The growth retardation of rice plants treated 1 day after transplanting with 100 g a.i./ha or more of the herbicide was highest among the 4 application timings, reaching 8% by visual evaluation at 14 days and was later reduced to zero (data not shown). Eventually, the rice plants suffered less than 10% reduction in dry weight (Fig. 4.1). There was no significant difference in dry

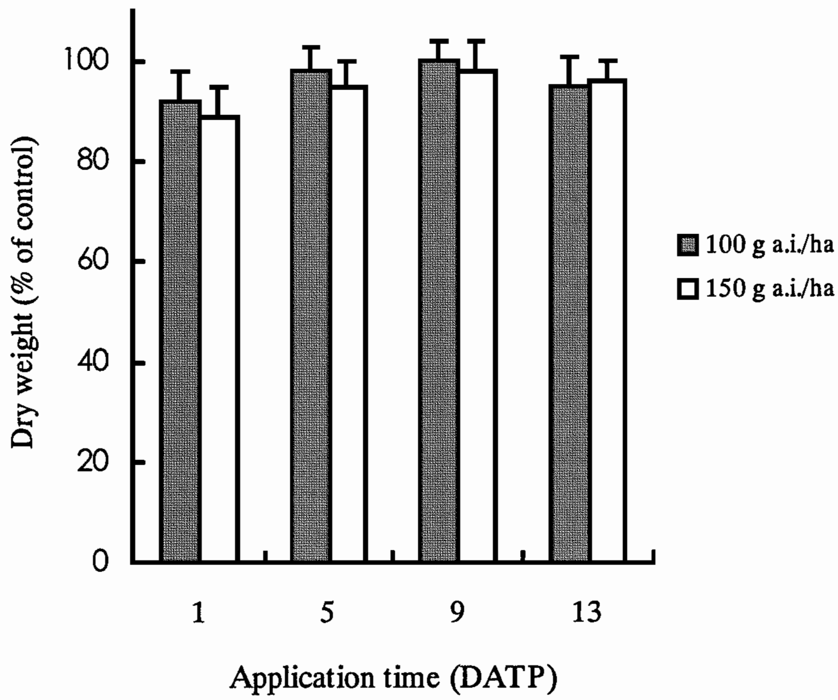


Fig. 4.1 Effect of application time on the phytotoxicity of imazosulfuron to rice plants transplanted at a 2 cm depth

Vertical bars represent standard deviation. Dry weight of the shoots was measured 35 days after the applications.

DATP : Days after transplanting

The dry weight of untreated control plants is as follows: 1 DATP, 3.86±0.13; 5 DATP, 4.77±0.25; 9 DATP, 6.96±0.31; 13 DATP, 8.33±0.69 g/hill weight between the transplanting times.

Effect of flooding water depth

Even applications of 150 g a.i./ha in the flooding water conducted at 1 cm or 5 cm depth did not result in growth retardation. The rice plants suffered less than 5% reduction in dry weight (Fig. 4.2), and there was no significant difference in dry weight between the two flooding water depths.

Effect of flooding water depth

Even at 150 g a.i./ha, dry weight of rice plants transplanted at a 2 cm depth was scarcely reduced by water leaching at the rate of 3 cm/day for 3 days (Fig. 4.3).

Effect of Transplanting Depth

Effect of transplanting depth on the phytotoxicity of imazosulfuron to rice plants was examined using 2 bioassay methods.

With an application of 75 g a.i./ha of imazosulfuron, the rice plants suffered no serious injury irrespective of differences in transplanting depth (Fig. 4.4). In case of shallow transplanting at a -0.5 cm depth, only slight growth retardation and reduction of the dry weight were observed at the higher dosage of 150 g a.i./ha. However, they did not result in any practical damage.

Application of imazosulfuron at 90 g a.i./ha caused the dry weight of plants transplanted at 0 cm and 1 cm depths to be 82% and 77% of the control, respectively (Fig. 4.5). These reductions caused by the growth retardation were significantly different from the untreated control. However, there was no significant difference in dry weight between the two transplanting depth. With pretilachlor application, however, the plants transplanted at 0 cm depth suffered much greater irrecoverable damage than those transplanting at 1 cm.

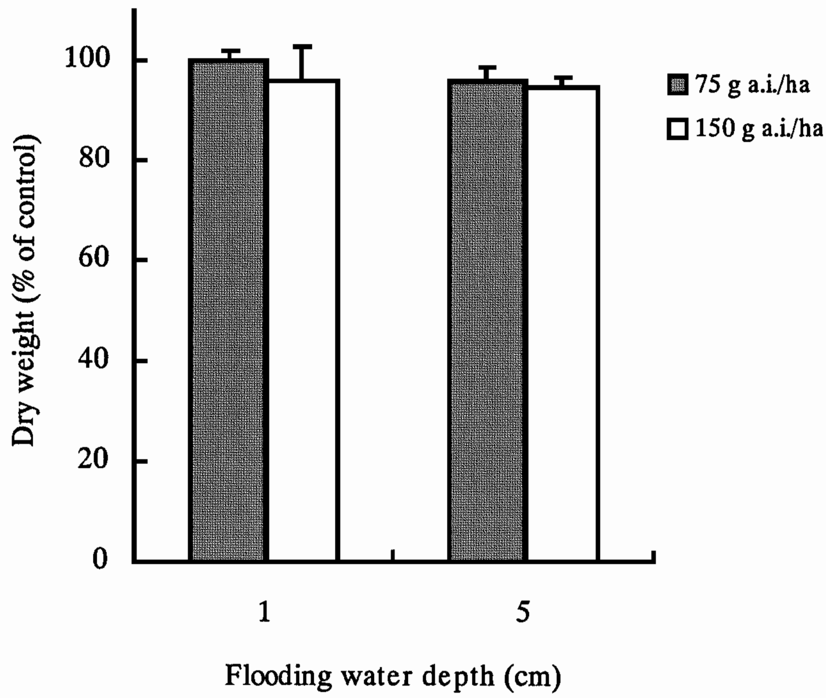


Fig. 4.2 Effect of flooding water depth on the phytotoxicity of imazosulfuron to rice plants transplanted at a 2 cm depth Vertical bars represent standard deviations.
The dry weight of untreated control is as follows:
1 cm flooding water depth, 1.25 ± 0.06 g/hill; 5 cm flooding water depth, 1.37 ± 0.15 g/hill

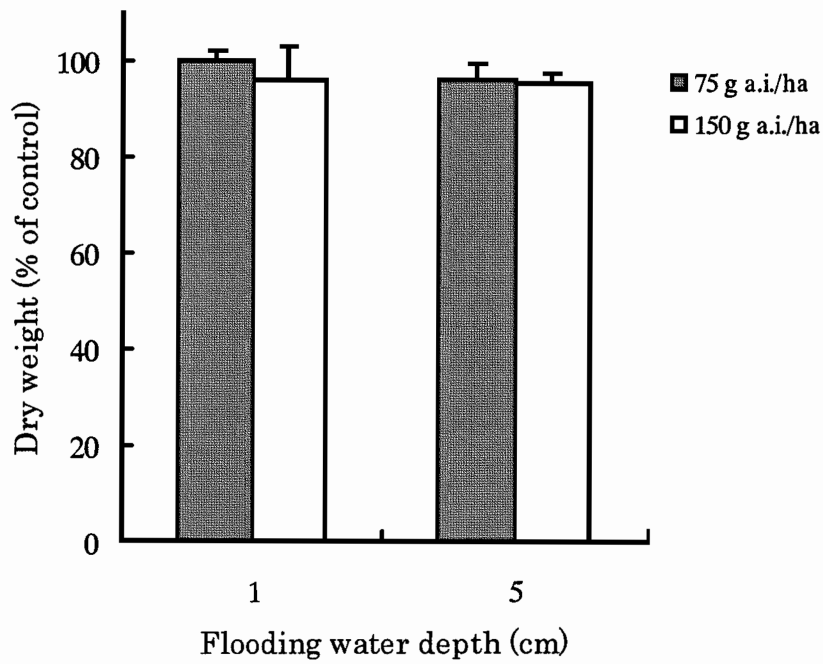


Fig. 4.3 Effect of water leaching on the phytotoxicity of imazosulfuron to rice plants
Vertical bars represent standard deviations.
The dry weight of untreated control plants is as follows:
no leaching, 2.83±0.22 g/hill; 3 cm/day leaching, 2.74±0.23 g/hill

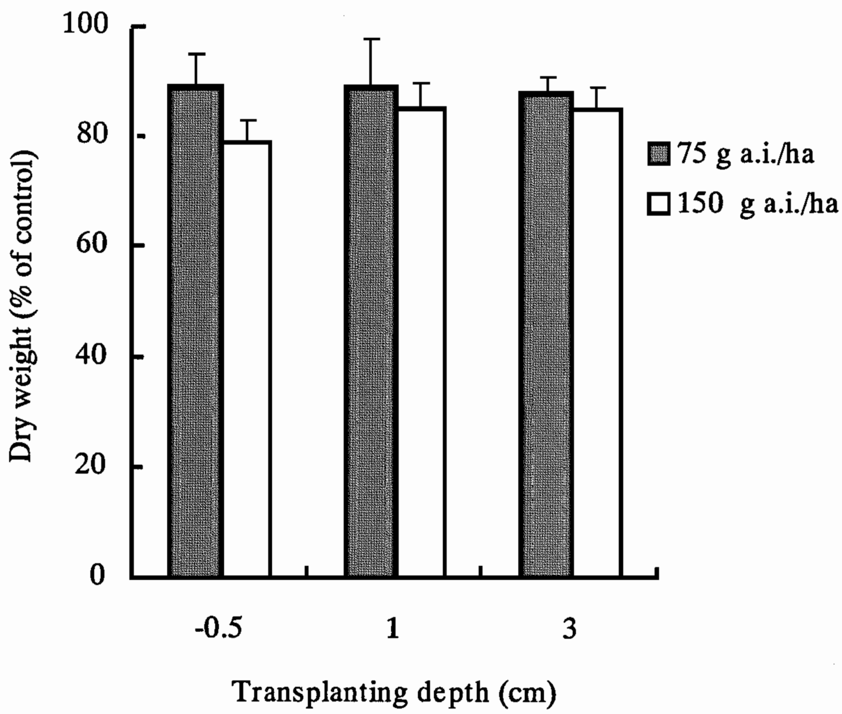


Fig. 4.4 Effect of transplanting depth on the phytotoxicity of imazosulfuron to rice plants
Vertical bars represent standard deviations.
The dry weight of untreated control is as follows:
-0.5 cm transplanting depth, 8.24 ± 0.32 g/hill; 1 cm transplanting depth, 8.65 ± 0.42 g/hill; 3 cm transplanting depth, 7.62 ± 0.50 g/hill

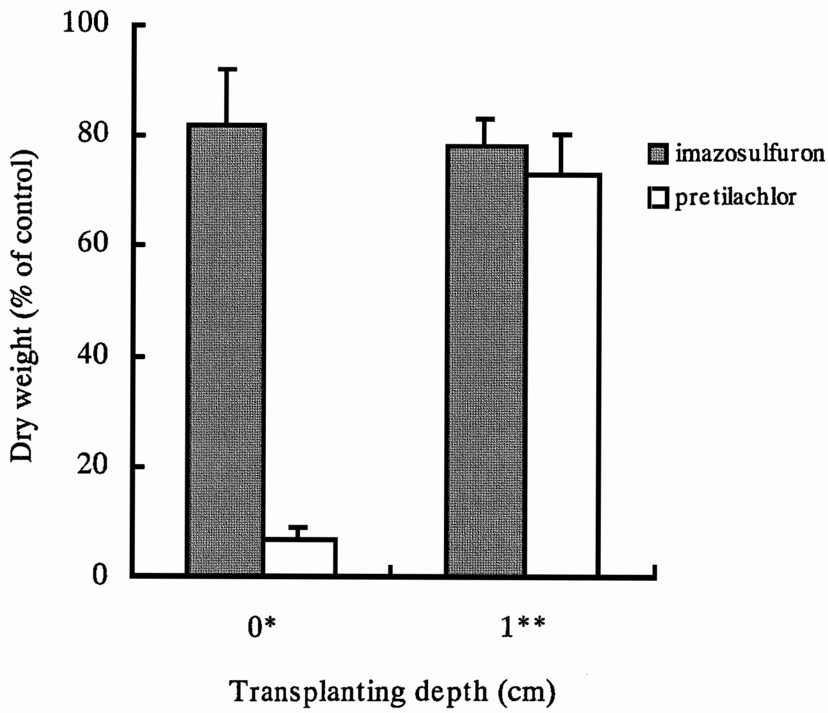


Fig. 4.5 Effect of transplanting depth on the phytotoxicity of imazosulfuron (90g a.i./ha) and pretilachlor (600 g a.i./ha) to rice plants

Vertical bars represent standard deviations.

*, Basal leaf sheaths of rice plants were exposed above the flooding water and the root tips were in the soil.

**, Root tips of rice plants were exposed above the flooding water and the basal leaf sheaths were in the soil.

The dry weight of untreated control plants is as follows:

0 cm transplanting depth, 3.45 ± 0.24 g/hill; 1 cm transplanting depth, 4.12 ± 0.36 g/hill

Effects of temperature and transplanting depth

The growth retardation of plants transplanted at the -0.5 cm depth was highest by visual evaluation, reaching 10% 14 days after the application and thereafter dropping (data not shown). Finally, the plants transplanted at the -0.5 cm depth suffered slightly more injury than those transplanted at the 2 cm depth. Among the lowest dry weight was found in those plants transplanted at -0.5 cm with the highest temperature after application (84% compared with the control); this was significantly different from the untreated control (Fig. 4.6). However, there was no significant difference in dry weight between the two depths under any temperature condition, nor was there any significant reduction in dry weight with temperature difference.

Effects of water leaching and transplanting depth

With water leaching at the rate of 2 cm/day for 3 days after application, no serious growth retardation or reduction of dry weight was observed irrespective of difference in herbicide dosage (100, 150 g a.i./ha) when rice plants were transplanted at a 3 cm depth; those plants transplanted at 1 cm depth, however, suffered greater injury in dry weight. Dry weight following application at 150 g a.i./ha, which was significantly different from the untreated control, was reduced more (77% compared with the control) than following 100 g a.i./ha. There was no significant difference in dry weight between the 1 cm and 3 cm transplanting depths, however (Fig. 4.7).

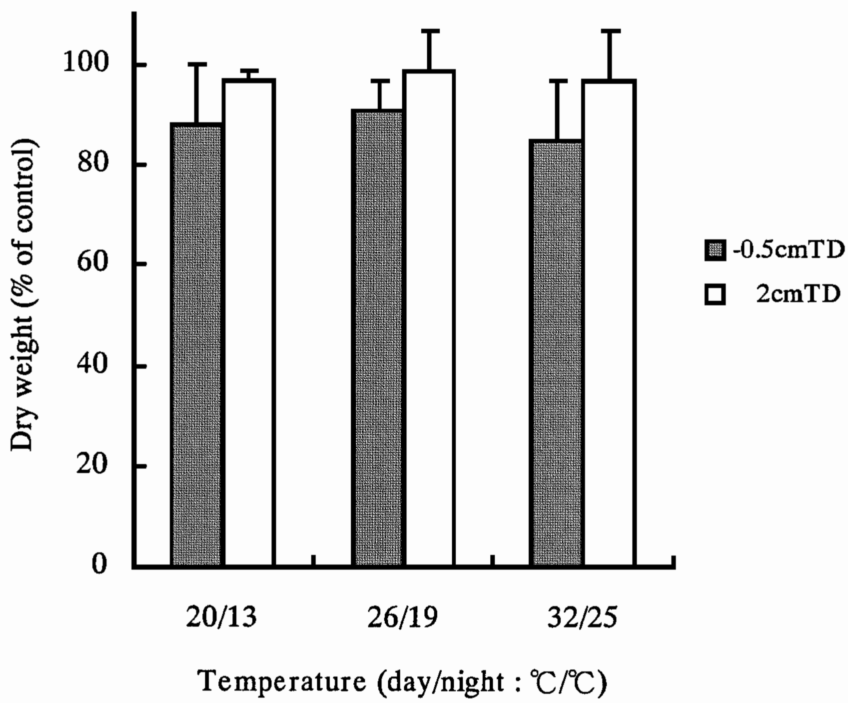


Fig. 4.6 Effect of temperature on the phytotoxicity of imazosulfuron (100 g a.i./ha) to rice plants transplanted at a -0.5 cm or a 2 cm depth

Vertical bars represent standard deviations.

-0.5 cm TD, Rice seedling planted at -0.5 cm depth; 2 cm TD, Rice seedling planted at 2 cm depth;

The dry weight of untreated control plants (20/13, 26/19. 32/25 ℃/℃) is as follows:

-0.5 cm transplanting depth, 1.72±0.23, 2.98±0.18, 4.18±0.38 g/hill; 2 cm transplanting depth, 1.50±0.19, 2.77±0.21, 3.81±0.24 g/hill

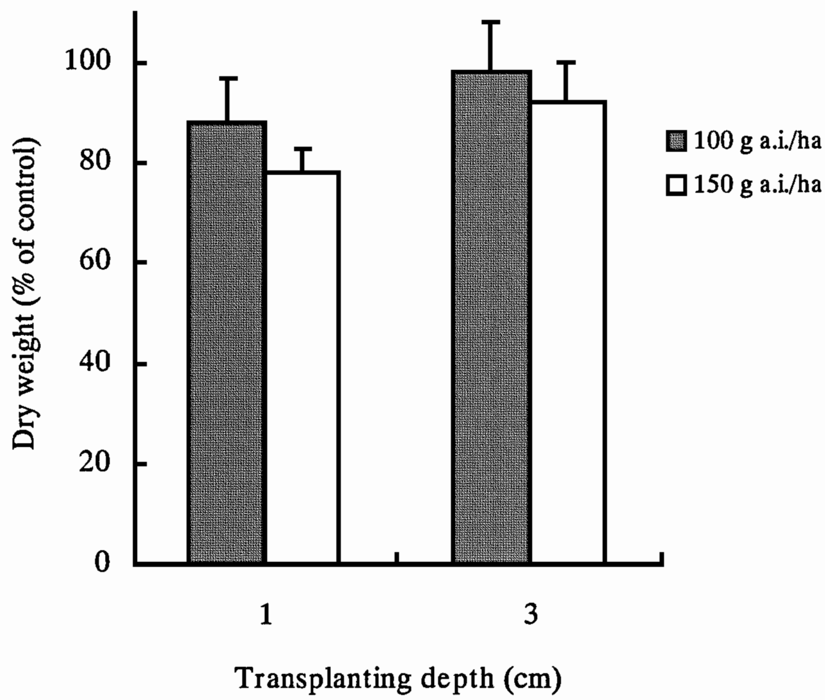


Fig. 4.7 Effect of transplanting depth under water leaching conditions on the phytotoxicity of imazosulfuron to rice plants

Vertical bars represent standard deviations.

The dry weight of untreated control is as follows:

1 cm transplanting depth, 1.07 ± 0.05 g/hill; 3 cm transplanting depth, 0.87 ± 0.12 g/hill

DISCUSSION

Early application timing of imazosulfuron slightly inhibited the growth of rice plants (Fig. 4.1), although this inhibition was later reduced to zero (data not shown). Plant age and growth rate affect response to a herbicide [13]. This means there may be a close relation among rooting development, age of the plants and the imazosulfuron phytotoxic activity on the plants.

Water flooding depth did not significantly affect the phytotoxic activity (Fig. 4.2); imazosulfuron was mainly absorbed by the roots and the basal leaf sheath of the plants (Chapter 3). Moreover, plant height with the flooding water application recovered earlier from the suppression than that with the soil incorporation or the soil application (Chapter 3). This means that the contact of imazosulfuron with the rice foliar plays no role in its phytotoxic action.

There was no significant difference in dry weight of rice plants irrespective of difference in transplanting depth with lower dosage of the application (Fig. 4.4). With higher dosage of the application, however, the dry weight of plants transplanted at -0.5 cm decreased the most; this was not significantly different from the dry weight of those transplanted deeper but was significantly different from untreated control (Fig. 4.4). Shallowly transplanted rice plants suffered growth retardation or significant reduction in dry weight with imazosulfuron application (Fig. 4.5). The underground parts (roots and basal leaf sheath) of shallowly transplanted plants can easily come into contact with the herbicide on the soil surface because of lack of protection by the soil. Imazosulfuron was absorbed by the roots and basal leaf sheath of the plants (Chapter 3). Growth inhibition was similar when either part was exposed to the herbicide (Fig. 4.5). These results suggest that imazosulfuron activity on rice plants primarily depends on its degree of contact with these underground plant parts.

Under the highest temperature conditions the shallowly transplanted rice suffered slight growth reduction. The paddy herbicide, butachlor, PCP, chlornitrofen and *s*-triazine herbicides are known to increase their activity under higher temperature conditions [27, 29]. Temperature response of pyrazosulfuron-ethyl was the opposite of these herbicides [12, 57]. Warm temperatures and high humidity often increases herbicidal action in relation to the following factors: 1. better absorption of the herbicides into plants, which is mainly depending on transpiring stream, 2. increase in the physicochemical reaction of the herbicides against plants cells under a high temperature, 3. change in the physiological and biochemical states of rice seedlings [27, 29]. Half life of imazosulfuron in excised leaves of crops and weeds was different [51] like other sulfonylureas [30, 43, 46]. The reason the phytotoxic activity of imazosulfuron increases under a higher temperature condition is not clear, but factors such as changes in absorption by the roots, change of biochemical processes in plant have been suggested.

Under the non-leaching condition, the dry weight of plants transplanted at the 1 cm depth was not reduced as much as those transplanted at 3 cm with the application of 150 g a.i./ha (Fig. 4.4). There was no significant difference in dry weight of rice plants transplanted at the 2 cm depth between non-leaching and water leaching with application of 150 g a.i./ha (Fig. 4.3). On the other hand, under the water leaching there was no significant reduction in dry weight of rice plants transplanted at a 2 cm depth but significant decrease in that transplanted at a 1 cm depth. These suggest that the water leaching rarely affects the growth of those transplanted at a 2 cm depth or more and treated with 150 g a.i./ha of imazosulfuron, whereas the greater the applied dosage of the herbicide and the shallower the transplanting depth of the plants, the more the water leaching inhibits their growth. Adsorption of imazosulfuron was dependent on soil types, and adsorption equilibrium reached in 2 hr. Imazosulfuron was hardly desorbed from the soil, and its mobility decreased with time irrespective of soil type [52]. These results supported that crop injury was highly related to transplanting depth and water leaching. Movement of imazosulfuron in paddy soil was limited to within 2 cm of the layer under the soil surface with water leaching after application using the bioassay method (Chapter 2). With pre-emergence application, water leaching and planting depth were the important factors influencing the activity of imazosulfuron (Chapter 2). These also suggest that the movement of imazosulfuron downward to the rooting zone of rice plants in the soil by water leaching brings the herbicide into contact with the underground parts (roots and basal leaf sheaths), and they absorb the herbicide, eventually leading to the growth inhibition of the plants. The early application timing, leaching conditions and shallow rice planting increased the growth inhibition caused by pyrazosulfuron-ethyl, and it is believed that this substance was mainly absorbed through the rice root system [12].

Consequently, the growth reduction of shallowly transplanted rice plants induced by imazosulfuron can be intensified by water leaching, early application time and higher temperature after application. These are very important factors affecting the phytotoxicity by imazosulfuron on plants shallowly transplanted depths.

SUMMARY

Application of imazosulfuron ranging from 75 g a.i./ha to 150 g a.i./ha caused no significant difference in dry weight of rice plants irrespective of variation in application time between 1 day and 13 days after transplanting or in flooding water depth between 1 cm and 5 cm. With an application of 75 g a.i./ha, rice plants suffered no serious injury irrespective of difference in transplanting depth. In case of shallow transplanting at a -0.5 cm depth, slight reduction of dry weight was observed at 150 g a.i./ha. No significant reduction in dry weight of rice plants transplanted at a depth between 1 cm and 3 cm. No

significant reduction in dry weight of rice plants transplanted at a depth of -0.5 cm or 2 cm was recognized irrespective of temperature (20℃/13℃, 26℃/19℃, 32℃/25℃; day/night) after treatment application of 100 g a.i./ha. There was no significant difference in dry weight between the -0.5 cm and the 2 cm transplanting depths under any temperature condition, although the dry weight of rice plants transplanted at the -0.5 cm depth under the highest temperature was significantly different from the untreated control. When either basal leaf sheaths or root tips of the rice plants were exposed to 90 g a.i./ha of imazosulfuron, the dry weights of the shoots were significantly different from untreated control. With water leaching after applications, the dry weight of rice plants transplanted at the 1 cm depth, which was significantly different from the untreated control, was reduced more than those transplanted at the 2 cm and more depth by application of 150 g a.i./ha.

CHAPTER 5

MODE OF ACTION

From both scientific and practical viewpoints, it is greatly important to elucidate the mode of action of herbicides. These tests enable compound with reduced environmental risk to be selected for further study. Sulfonylurea inhibits ALS [31-34], an important enzyme in the pathway for biosynthesis branched-chain amino acids. Alleviative effects of branched-chain amino acids on the growth inhibition by imazosulfuron were investigated using rice plants. Additionally, the inhibitory effects on ALS prepared from 6 plants were examined. Investigation for inhibition properties of imazosulfuron on ALS was carried out using pea ALS.

MATERIALS AND METHODS

Effect of imazosulfuron on rice plant growth

Germinated seeds of rice plants (Oryza sativa cv. Nipponbare, ORYSA) were planted on a float made of plastic in a 500-ml plastic beaker filled with 250 ml of Kimura B nutrient solution and were grown in a growth chamber at 28℃ and 16-h daylength at 20,000 lux. Seedlings at the two-leaf stage were transferred to a nutrient solution containing imazosulfuron at concentrations between 1 and 100 ppb. The lengths of the third, fourth and fifth leaves and the dry weight of the roots were measured 13 days after treatment.

Effects of valine, isoleucine and leucine on growth inhibition of the third leaf of rice plants induced by imazosulfuron

Rice plants were grown using the nutrient solution described above. Seedlings at the two-leaf stage were treated with the nutrient solution containing 30 or 100 ppb of imazosulfuron with or without 10, 30 or 100 ppm each of valine, isoleucine and leucine or 100 ppm of valine and isoleucine. The length of the third leaf was measured 4 days after treatment.

Preparation of ALS

The procedure used in this study was a modification of that employed by Ray [31]. ALS was prepared from the etiolated shoots of pea (*Pisum sativum*, PISSA), ECHOR, SAGPY, CYPSE and wheat (*Triticum aestivum*, TRIAE) ranging from 14 and 18-day-old, and from 5-, 12- or 19-day-old shoots of rice plants grown in a growth chamber at 28℃ and 24-h daylength at 20,000 lux or in the dark. The shoots of the plants were homogenized in 3 volumes of buffer containing 0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine-pyrophosphate (TPP), 10 mM flavine adenine dinucleotide (FAD) and 10 % v/v glycerol. The homogenate was filtered through 8 layers of gauze and centrifuged at 27,000g for 20 min. ALS was collected at 25-50 % saturation with $(NH_4)_2SO_4$ at 17,000g for 30 min by centrifugation. The pellet was then dissolved in buffer containing 0.1 M K_2HPO_4, pH 7.5, 20 mM pyruvate and 0.5 mM $MgCl_2$ and desalted on a Sephadex G-25 (Pharmacia PA-10) column equilibrated with the same buffer. The desalted enzyme was used immediately for assays. All these manipulations were done at 0 to 4℃.

Assay of ALS

ALS assays were carried out in a final volume of 1.3 ml at 30℃. The final reaction mixture contained 20 mM K_2HPO_4, pH 7.0, 20 mM sodium pyruvate, 0.5 mM TPP, 0.5 mM $MgCl_2$, 10 mM FAD, and various concentrations of imazosulfuron. The herbicide was dissolved in acetone and diluted with phosphate buffer. Final concentration of the acetone in the reaction mixture was kept to less than 0.2 % (v/v). Assays were initiated by adding enzyme (100 ml) and terminated by adding 100 ml of 50 % H_2SO_4. Acetolactate was determined as described by Westerfield [60] with the following modifications. The acidified reaction mixture was heated for 20 min at 40℃ after 1 ml of 0.5 % w/v creatine was added; 1 ml of 5 % α-naphthol, freshly prepared in 10 % NaOH, was then added. After mixing, the solution was incubated at room temperature for 1 h. The absorbance was then measured spectrophotometrically at 530 nm. Protein was determined by the method of Lowry *et al.* [61]. The concentration required to inhibit enzyme activity by 50 % (I_{50}) was calculated.

Inhibitory properties of imazosulfuron with pyruvate or TPP on ALS

All procedures in the preparation and assay of ALS were as described above with the following modifications. ALS prepared from PISSA was used. TPP concentration in the reaction mixtures was kept at 0.5 mM when the pyruvate concentration was varied between 4 and 20 mM for the inhibitory property of imazosulfuron with pyruvate, while the pyruvate concentration was kept at 20 mM when the TPP concentration was varied between 12.5 and 400 mM for the inhibitory property of imazosulfuron with TPP. An imazosulfuron concentration ranging from 10 to 30 nM was used. The Ki values were calculated using a Hanes-Woolf plot [62].

Time Course of ALS Activity in the Presence of Imazosulfuron

All procedures in the preparation and assay of ALS were the same method in Section 2. ALS prepared from 14-day-old etiolated PISSA was used. The enzyme activity in the assay buffer was measured after an incubation time of

10, 20, 30, 60, 90, 150, 210, 270, 330, 390 or 450 min with concentrations between 1 and 10 nM of imazosulfuron. Initial inhibition was estimated using a 10-min assay. The inhibition constants for the final, steady-state inhibition were determined using a time interval from 390 to 450 min. Ki values were determined as 3 nM for imazosulfuron. Calculation was done by the method of LaRossa and Schloss [63].

RESULTS

Effect on rice plants growth

Leaf length of rice plants was inhibited by 30 ppb or more of imazosulfuron, whereas root dry weight was reduced at concentrations of 1 ppb and above (Fig. 5.1). Growth inhibition of leaves and roots by imazosulfuron was more apparent at the highest concentration of 100 ppb. At this concentration, the fourth leaf length and the dry weight of the roots were reduced to 50% of the control.

Effects of valine, isoleucine and leucine on growth inhibition of the third leaf of rice plants induced by imazosulfuron

Imazosulfuron at 30 or 100 ppb inhibited the third leaf growth of rice plants by 28% and 44%, respectively (Fig. 5.2). The addition of valine, isoleucine and leucine reversed the inhibition more when they were applied at higher concentrations. These amino acids, at a concentration of 100 ppm each, alleviated the growth inhibition of the third leaf induced by 30 ppb of imazosulfuron to almost the same degree as that of the control; however, it did not completely reverse that induced by 100 ppb of the herbicide. The alleviation effect of the three amino acids was much higher than that of just the

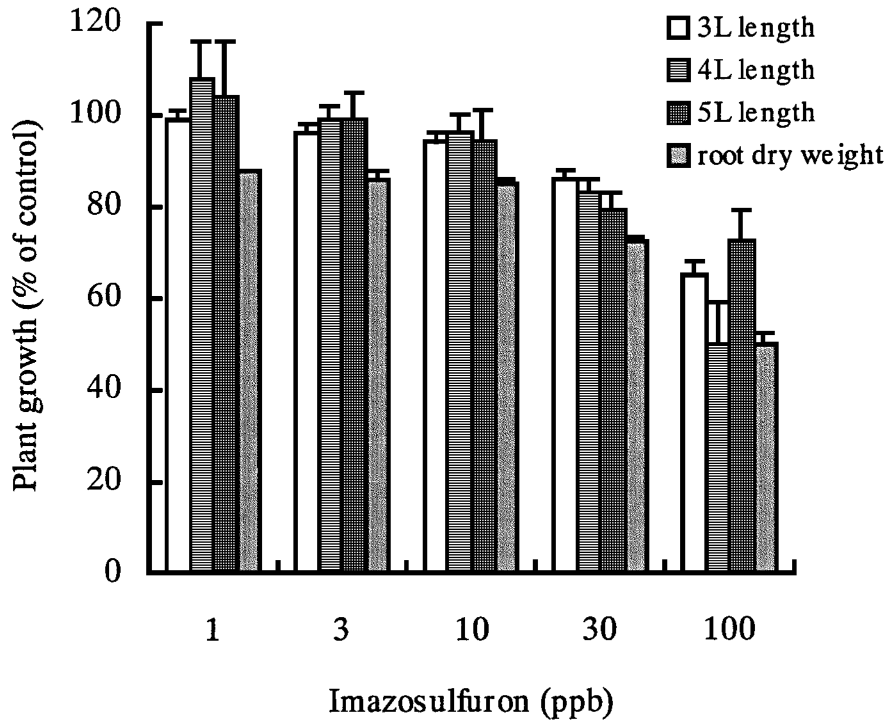


Fig. 5.1 Effect of imazosulfuron on growth of rice plants
Data are means of three replicates. Vertical bars represent ±SD.
L, leaf stage

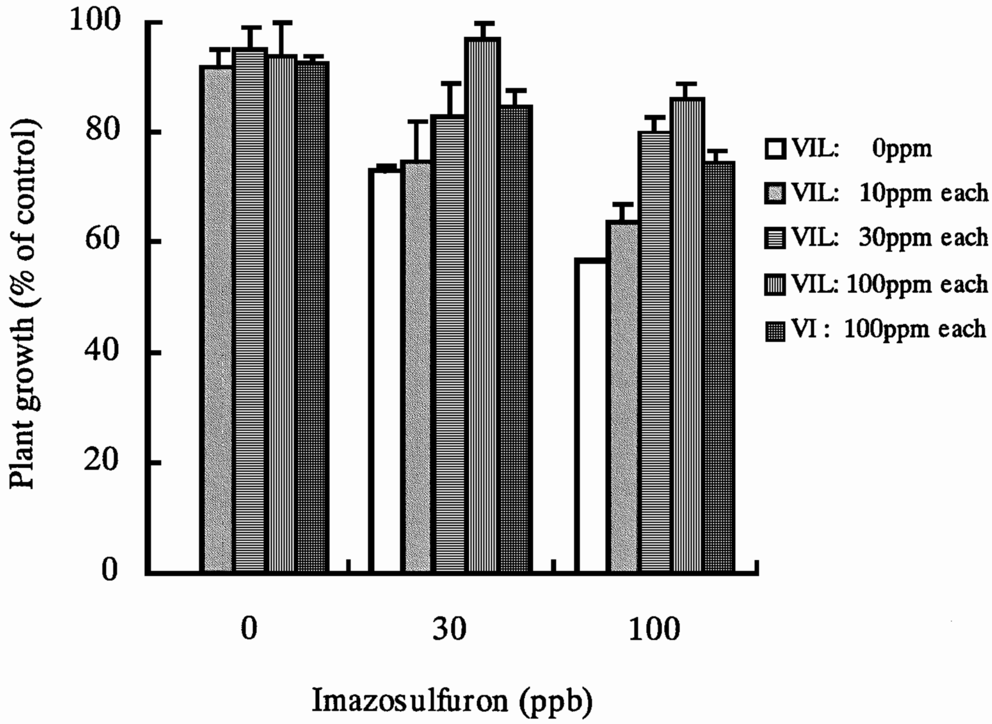


Fig. 5.2 Effect of valine, isoleucine and leucine on the growth inhibition of the third leaf of rice plants induced by imazosulfuron

Data are means of three replicates. Vertical bars represent ± SD.
V, valine; I, isoleucine; L, leucine

two, valine and isoleucine.

Inhibition of plant ALS

I_{50} for ALS from ECHOR, SAGPY, CYPSE and PISSA which were quite sensitive to imazosulfuron was 20 to 58 nM, while that from rice plant and wheat which were tolerant was 14 to 19 nM (Table 5.1).

Inhibition of ALS from rice plants under various growth conditions

ALS activity and the effect of imazosulfuron on the enzyme activity at various age or chloroplast development in rice plants was examined. Table 5.2 lists I_{50} values of imazosulfuron for ALS prepared from rice plants, which are tolerant to imazosulfuron, grown for 5 to 19 days in the dark and the light. The 12-day-old shoots grown in the dark showed the highest specific activity of ALS, followed by 12-day-old in the light, 5-day-old in the dark, 5-day-old in the light and 19-day-old in the light. The I_{50} value decreased when the ALS activity increased. The ALS activity which chloroplast development of rice plants enhances did not overcome the inhibition induced by imazosulfuron.

Inhibitory properties of imazosulfuron on acetolactate Synthase

Inhibition of ALS from PISSA by imazosulfuron was examined at different concentrations of pyruvate as a substrate and TPP as a cofactor. At concentrations between 4 and 20 mM of pyruvate, imazosulfuron showed noncompetitive inhibition with respect to pyruvate as shown in the Hanes-Woolf plot (Ki slope, 20 nM; Ki intercept, 24 nM) (Fig. 5.3). A Km value of 1.2 mM for pyruvate was obtained. At concentrations between 12.5 and 400 mM of TPP, imazosulfuron showed uncompetitive inhibition with respect to TPP (Ki slope, 33 nM; Ki intercept, 19 nM) (Fig. 5.4). The Km value for TPP was 6 mM.

Table 5.1 Inhibition of acetolactate synthase isolated from plants by imazosulfuron

plant	I_{50}*	ALS activity**
ECHOR	26	98
SAGPY	58	80
CYPSE	20	89
TRIAE	19	116
ORYSA	14	412
PISSA	24	610

* : The I_{50} values represent the concentrations of imazosulfuron required to inhibit acetolactate synthase activity 50%.
** : Acetolactate synthase activity (nmol acetoin / hr / mg protein)

Table 5.2 Inhibition of acetolactate synthases from rice plants under various growth conditions by imazosulfuron

Growth condition		I_{50}* (nM)	ALS activity**
light	5-day-old	32	251
	12-day-old	21	314
	19-day-old	45	215
dark	5-day-old	22	281
	12-day-old	14	412

* : The I_{50} values represent the concentrations of imazosulfuron required to inhibit acetolactate synthase activity by 50%.
** : Acetolactate synthase activity (nmol acetoin / hr / mg protein)

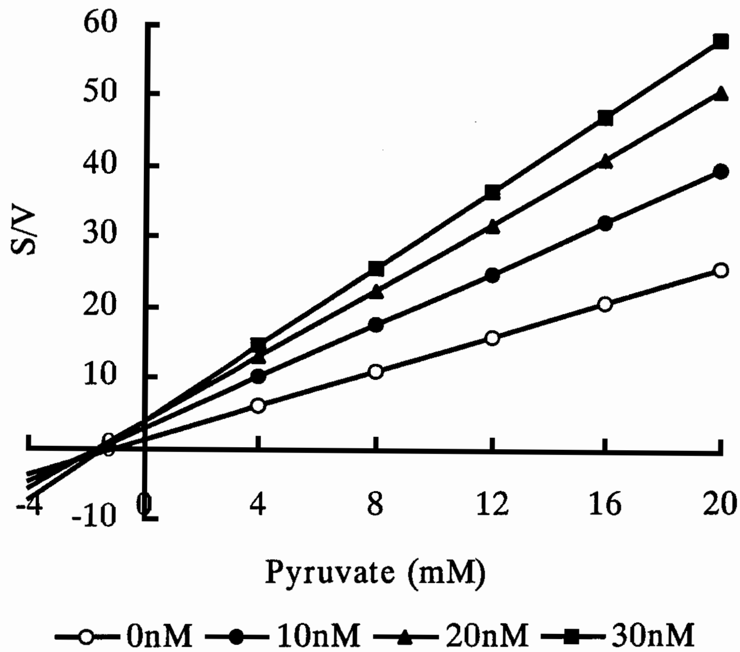


Fig. 5.3 Inhibitory properties of imazosulfuron (0, 10, 20, 30 nM) with pyruvate on the acetolactate synthase activity of PISSA

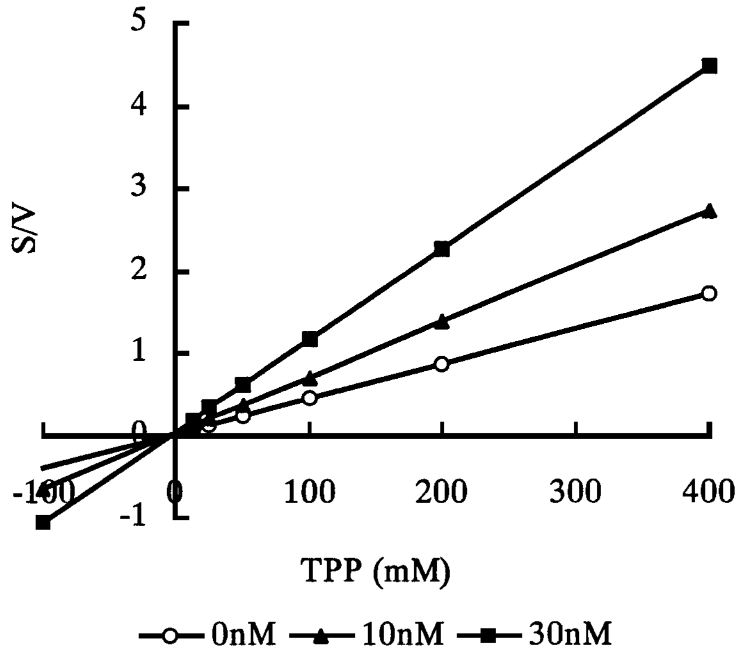


Fig. 5.4 Inhibitory properties of imazosulfuron (0, 10, 30 nM) with TPP on acetolactate synthase activity of PISSA

Time course of acetolactate synthase activity in the presence of imazosulfuron

The progress curves for inhibition of PISSA ALS activity indicated that the amount of inhibition increased with longer incubation time when the enzyme was assayed in the continued presence of imazosulfuron (Fig. 5.5). Assay progress curves were adequately defined by first-order transients in which there were both an initial level and a final, steady-state level of inhibition. ALS activity of the control without imazosulfuron was linear up to 450 min. Initial and final Ki values were determined as 81 nM and 4 nM, respectively. The maximum rate constant for the (time-dependent) transition between the initial and the final, steady-state inhibition by imazosulfuron was estimated to be approximately 0.052 min^{-1}.

DISCUSSION

Imazosulfuron seems to inhibit growth of the shoots and roots of rice plants and that the inhibition of root elongation is stronger. Similar results were observed in chlorsulfuron [31], DPX-T6375 [11] and bensulfuron-methyl [11]. As described in Chapter 3, growth inhibition of rice plants with the soil or soil incorporation applications of imazosulfuron was greater than that with the flooding water application. These are consistent with the sensitivity of rice plant roots to imazosulfuron being higher than that of the shoots.

Branched-chain amino acids alleviated the growth inhibition of rice plants induced by imazosulfuron. Shimizu *et al.* indicated that the addition of these amino acids and the precursors of the valine-isoleucine pathway below that of acetolactate formation, α-ketoisovalerate and α-keto-β-methylvalerate, alleviated the growth inhibition of pea root tips induced by imazosulfuron, while the precursors above the acetolactate, as pyruvate and threonine, were

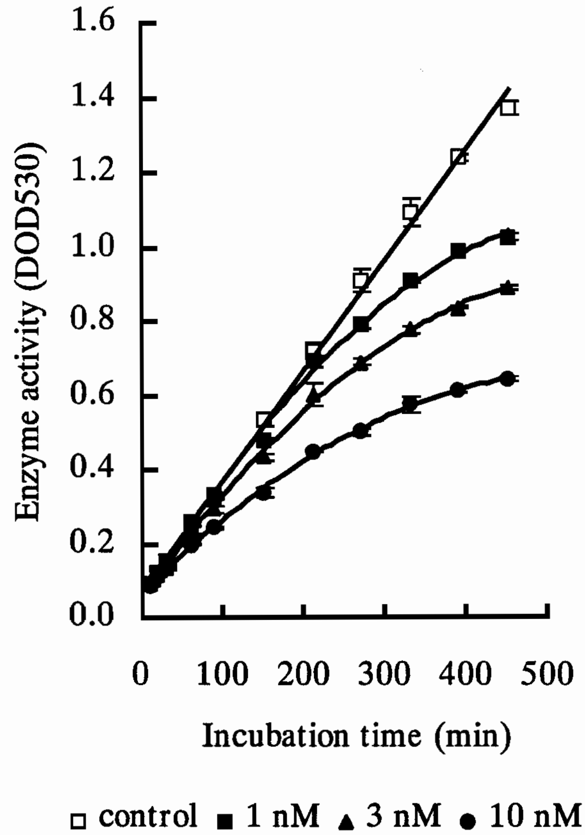


Fig. 5.5 Time course of acetolactate synthase activity of PISSA in the presence of imazosulfuron (1, 3, 10 nM)
Data are means of three replicates. Vertical bars represent ± SD.

not effective [64]. Therefore, imazosulfuron seems to inhibit the biosynthetic pathway of the branched-chain amino acids.

Addition of valine, isoleucine and leucine reversed growth inhibition of rice plants induced with imazosulfuron (Section 1). I_{50} for ALS from both sensitive and tolerant plants was 14 to 58 nM. These results strongly support that imazosulfuron acts on plant tissue by directly inhibiting the enzyme ALS which catalyzes the first step in the biosynthetic pathway of the branched-chain amino acids as well as the other ALS inhibitors [31-34].

The chlorsulfuron-insensitivity of the legume plants at early growth stage after germination depended on the contents of both ALS and acetoin forming enzyme which was involved in regulating the supply of amino acids for storage of protein synthesis [47]. The ALS did not exist in the cotyledon in the early days following germination [47]; however, ALS which is sensitive to imazosulfuron did exist in rice plants in the early growth stage after germination. Therefore, the insensitivity of rice plants to imazosulfuron is probably not due to the enhancement of ALS activity to age and chloroplast development, or to a lack of ALS at the early growth stage.

The ALS from a wide variety of plant species, both tolerant and sensitive, was sensitive to sulfonylurea [31]. The I_{50} value for ALS from 3 paddy weeds which were quite sensitive to imazosulfuron was 20 to 58 nM (Table 5.1), while that from rice plant which was tolerant was 14 to 45 nM (Table 5.2). The author also described in Chapter 2 that I_{50} value of imazosulfuron for intact plants of these 3 weeds ranged from 487 to 2218 times less than that for rice plants applied 7 days after transplanting. The morphology of a plant can determine the selectivity between rice plants and paddy weeds. Morphological differences are found in root systems, location of growing points and uptake parts [13]. Most paddy weeds can be easily controlled because of shallow root systems, whereas rice plants have deep, extensive root systems. Such deep root systems may decrease crop injury from imazosulfuron that remains near the soil surface through depth-protection. In CYPSE imazosulfuron was mostly absorbed by root and partly by shoot, while in rice plants by both root and basal leaf sheaths (Section 3). Shoots parts in broadleaf weeds that are important for herbicide uptake are the hypocotyl, epicotyl and cotyledon, whereas the most important shoot parts in grasses are the coleoptile and mesocotyl [17]. This factor may be based on the selectivity between rice plants and broadleaf weeds. In addition to morphological factor, biochemical factor also affects the selectivity. Kamizono *et al.* reported that the half life of imazosulfuron in excised leaves of rice plants and CYPSE was 4.0-5.1 hr and 25.8-35.2 hr, respectively [51].

Accordingly, the contrast between I_{50} values for the intact plants and for ALS indicate that the selectivity of imazosulfuron does not seem to be due to the difference in the enzyme level reaction with ALS. The difference in the metabolic rate of imazosulfuron between rice plants and CYPSE may cause selectivity between them like other sulfonylureas.

Imazosulfuron showed noncompetitive inhibition with respect to pyruvate and uncompetitive inhibition to TPP. It has been shown that sulfonylurea herbicides such as chlorsulfuron [39-41], triazolopyrimidine sulfonamide [44] and pyrimidinyl salicylic acid compounds [65] inhibited ALS activity from the plants in a noncompetitive or mixed-type manner with respect to pyruvate. Nakata [41] and Subramanian *et al.* [66] reported that the inhibition types of chlorsulfuron and triazolopyrimidine sufonamide were uncompetitive inhibitors of TPP. Close proximity on the ALS between sulfonylurea herbicide, TPP and FAD was suggested [67]. It has been proposed that the herbicides bind most tightly to the FAD enzyme complex and interfere with the binding of the keto acid molecule [67]. Considering these results and information, I suggest that imazosulfuron does not overlap with the site on ALS where pyruvate or TPP bind but inhibits the enzyme activity by binding the complexes of the enzyme, cofactor and substrate as do other sulfonylurea [39-41] and triazolopyrimidine sulfonamide [66, 68] herbicides.

Similar inhibition kinetics was observed with purified bacteria enzyme suggesting that the mechanism of inhibition of ALS was similar in plants and bacteria [31, 63].

Consequently, imazosulfuron is thought to be a slow-binding inhibitor as are other ALS inhibitor, such as sulfometuron methyl [63] and imazapyr [69]. Moreover, the maximal first order rate constant for transition from the initial to the final steady state inhibition of imazosulfuron was nearly identical to that of chlorsulfuron using the ALS from barley [39]. This biphasic feature can be explained by a conversion of an initial and rapidly formed ALS-imazosulfuron complex to a more tightly bound complex.

SUMMARY

The elongation of the third, fourth and fifth leaves of rice plants was inhibited when 30 ppb or more of imazosulfuron was contained in nutrient solution when the plants were treated at the two-leaf stage, whereas root dry weight was reduced at concentrations of 1 ppb and above.

Addition of the branched-amino acids, valine, isoleucine and leucine at a concentration of 100 ppm each alleviated the growth inhibition of the third leaf of rice plants induced by 30 ppb of imazosulfuron to almost the same degree as the elongation of the control.

Acetolactate synthase (ALS) activity prepared from both sensitive and tolerant plants to imazosulfuron was inhibited to the degree 50% at a concentration ranging from 14 to 58 nM and that from rice plants which were tolerant to imazosulfuron was also sensitive to the herbicide irrespective of age or chloroplast development of the plants (I_{50} = 14 to 45 nM). The ALS activity prepared from PISSA was inhibited in noncompetitive with respect to pyruvate and in uncompetitive with respect to thiamine pyrophosphate (TPP). Inhibition of ALS activity by the continuous presence of imazosulfuron was time dependent and biphasic. The constant of the initial inhibition by the herbicide was 20-fold larger than that of the final steady-state.

The results suggest that imazosulfuron acts on the plant by blocking the biosynthesis of valine, isoleucine and leucine, which is due to the direct inhibition of ALS. The herbicide is a slow-binding inhibitor of ALS activity. The binding site of imazosulfuron on the enzyme is judged not to overlap with that of pyruvate and TPP. Tolerance of rice plants to imazosulfuron does not depend on the sensitivity of ALS activity irrespective of the difference in plant age or growth condition.

CHAPTER 6

PHYSIOLOGICAL RESPONSES OF *Cyperus serotinus* Rottb. TO IMAZOSULFURON IN COMBINATION WITH VALINE, ISOLEUCINE AND LEUCINE

Although the biochemical site of imazosulfuron was identified as previously described, the connection among inhibition of ALS, the rapid cessation of plant division and general growth inhibition remains an area of active research. This chapter describes the physiological effect of branched-chain amino acids on the growth inhibition induced by imazosulfuron and the inhibition of imazosulfuron on protein, RNA and DNA synthesis, soluble protein levels, free amino acid levels using CYPSE.

MATERIALS AND METHODS

Effect of Valine, Isoleucine and Leucine on Inhibitory Action of Imazosulfuron

Tubers of CYPSE were placed on a chemical cloth saturated with water in a stainless steel case and grown in the dark at 28℃ for 3 days. Sprouted tubers were transferred to a 2000-ml plastic case containing 1000 ml of Kimura B nutrient solution at pH 5.5, and grown in a controlled growth chamber at 28℃ with a 16-hr photoperiod and a 20,000-lux illuminance for 4 days.

CYPSE grown as described above was placed in a 500-ml plastic beaker containing 250 ml of Kimura B nutrient solution. Imazosulfuron was added to the nutrient solution to give various final concentrations with or without valine, isoleucine and leucine. Plant height was measured after 14 days, and the experiment was repeated three times.

Protein, RNA and DNA synthesis measurements

Measurements of the effects of imazosulfuron on protein, RNA and DNA synthesis were done by determining the incorporation of tritium (^3H)-labeled precursors into the appropriate metabolic product. The radiolabeled material consisted of [^3H]leucine (sp. act. 1.48-2.22 TBq/mmol, 37 kBq/ml) for protein synthesis, [^3H]uridine (sp. act. 1.29-1.85 TBq/mmol, 37 kBq/ml) for RNA synthesis and [^3H]thymidine (sp. act. 740 GBq/mmol, 37 kBq/ml) for DNA synthesis. All measurements were done on 2-cm root tips excised from CYPSE. The plants grown as described above were treated with imazosulfuron contained in the nutrient solution. At the indicated time, root tips were excised just before they were used to measure the incorporation of the radiolabeled precursors into their respective metabolic products. The incubation medium contained 10 mM potassium phosphate buffer, pH 6, 1% sucrose, 2 mg streptomycin sulfate and 37 kBq of radiolabeled precursor per 5 ml. The roots were incubated for 1 hr at 28℃ with constant shaking. The incorporation of radiolabeled leucine, uridine and thymidine into protein, RNA and DNA, respectively, was measured by the procedures of Rost and Bayer [70]. The root tips were washed three times with cold, unlabeled incubation medium and then ground in 5 ml of cold (4℃) 80% ethanol. This extract was filtered through a GF/C glass fiber. The filter was washed successively with 15 ml each of 80% ethanol, 5% trichloroacetic acid, ethanol : diethyl ether (1:1, v:v) and diethyl ether to measure its incorporation in TCA-precipitable material. The filter was dried at room temperature and then placed in a scintillation vial with 10 ml of a scintillation cocktail so that the amount of radiolabeled material on the filter could be determined. The amount of radiolabeled material in the initial filtrate was also measured to determine the total amount of radiolabeled precursor that was absorbed by the excised root tips. The results were expressed as the mean of 3 replications.

Effects of Valine, Isoleucine and Leucine on Inhibitory Action of Imazosulfuron on DNA Synthesis

CYPSE grown as described above was placed in the nutrient solution containing 10 ppb of imazosulfuron simultaneously with 300 ppm each of valine, isoleucine and leucine. Roots were excised at the terminal 2-cm of root tips 4 hr after treatment. DNA synthesis was measured by the same method as previously described.

In time course studies of the effects of these amino acids, CYPSE grown as described was placed in nutrient solution containing 10 ppb of imazosulfuron for 24 hr and retreated with either 10 ppb imazosulfuron or 10 ppb imazosulfuron plus 300 ppm each of valine, isoleucine and leucine. At the indicated time, the roots were excised and then DNA synthesis was measured as described above. The results were expressed as the mean of 3 replications.

Soluble protein levels

CYPSE grown as above was placed in nutrient solution containing 3, 10, 30 or 100 ppb of imazosulfuron and then harvested 1, 4, 8 or 11 days after treatment. Roots were excised at the terminal 2-cm of root tips. The level of soluble proteins was determined by grinding 100 mg of root tips in 5 ml of 10 mM potassium phosphate buffer (pH 6.0). The extract was centrifuged at 20,000g for 15 min and the content of soluble protein in the supernatant was determined by the method of Bradford [71].

Free amino acid levels

Total free amino acid content was determined by grinding 100 mg of root tips in 5 ml of 5% trichloroacetic acid, centrifuging the extract at 20,000g for 15 min and measuring the content of the amino acids in the supernatant fraction by ninhydrin reaction [72]. These experiments were replicated three times.

RESULTS

Effects of valine, isoleucine and leucine on inhibitory action of imazosulfuron

Elongation of plant height of CYPSE was significantly reduced by treatment with a concentration of 1 ppb or more of imazosulfuron and with 10 ppb was reduced to 33% of control (Fig. 6.1). The concentration causing 50% reduction in elongation of plant height was 3.4 ppb.

The reduction in the elongation of the plant height was largely alleviated by adding valine, isoleucine and leucine to the nutrient solution containing the herbicide. The amount of the reversal from the reduction in plant height induced by 3 ppb or more of imazosulfuron increased further but that induced by 1 ppb of the herbicide slightly decreased when amino acids were simultaneously added at higher concentrations.

The elongation of plant height was slightly reduced by treatment with 300 ppm of each of the amino acids, however, this was not significant. Similar reduction was also observed when 300 ppm of amino acids was added to the solution containing 0.3 ppb or less of imazosulfuron. The elongation alleviated by the treatment of 300 ppm of each of the amino acids simultaneously with the herbicide did not exceed that by the treatment of this amount of each amino acid alone.

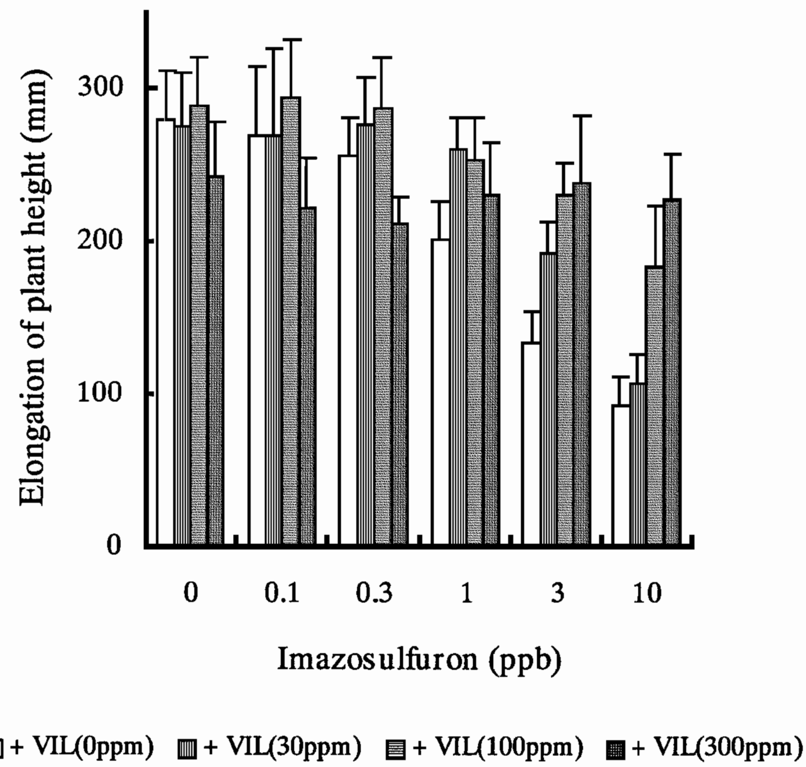


Fig. 6.1 Effect of valine, isoleucine and leucine on the inhibitory action of imazosulfuron on plant height of CYPSE

Vertical bars represent standard errors of the mean.

VIL : valine, isoleucine and leucine

Effect of imazosulfuron on protein, RNA and DNA synthesis

Uptake of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine was not inhibited 4 hr after treatment with 10 or 100 ppb of imazosulfuron (Table 6.1). Protein synthesis measured by leucine incorporation was also not inhibited by 10 or 100 ppb of the herbicide after 4 hr, while RNA and DNA synthesis measured by uridine and thymidine incorporation were significantly inhibited compared with the control. One way to separate the effect of imazosulfuron on uptake of a radiolabeled precursor from the effect on the incorporation of the precursor into its metabolic product is to compare the ratio of the amount of radiolabeled material incorporated into a metabolic product to the amount of radiolabeled material absorbed by the tissue. Imazosulfuron significantly reduced the proportion of thymidine and uridine incorporated into DNA and RNA, respectively, but had no significant effect on the amount of leucine incorporated into the protein. Treatment with 10 and 100 ppb of imazosulfuron reduced RNA synthesis measured by [^{3}H]uridine incorporation to 82% and 77% of the control, respectively, and DNA synthesis measured by [^{3}H]thymidine incorporation to 51 and 52% after 4 hr.

Treatment with 10 ppb of imazosulfuron significantly reduced the proportion of thymidine incorporated into DNA to 65% of the zero time value of radioactivity 1 hr after treatment and to 28% after 6 hr. Imazosulfuron also reduced the proportion of thymidine uptake with increasing length of imazosulfuron treatment and reduced that of thymidine incorporation into DNA to 45% (incorporation / uptake) after 6 hr (Fig. 6.2).

Effects of Valine, Isoleucine and Leucine on Inhibitory Action of Imazosulfuron on DNA Synthesis

All three amino acids increasingly alleviated DNA synthesis inhibited by 10 ppb of imazosulfuron 4 hr after treatment with higher concentration of amino

Table 6.1 Effect of imazosulfuron on protein, RNA and DNA synthesis in 7-day-old CYPSE root tips

	Treatment [ppb]	Uptake [Bq]*	Incorporation [Bq]*	Ratio**	% of Control
DNA	0	1710	252	0.15	100
$[^3H]$Thy	10	1988	152 a	0.08 a	51 a
	100	1885	145 a	0.08 a	52 a
RNA	0	4988	599	0.13	100
$[^3H]$Uri	10	4811	494 a	0.10 a	82 a
	100	4434	427 a	0.10 a	77 a
Protein	0	18068	8735	0.48	100
$[^3H]$Leu	10	16495	8130	0.49	102
	100	19402	8735	0.45	93

*, Bq / 100 mg fresh weight of root tips
**, Incorporation / Uptake
a, Significantly different from the control at P=0.05

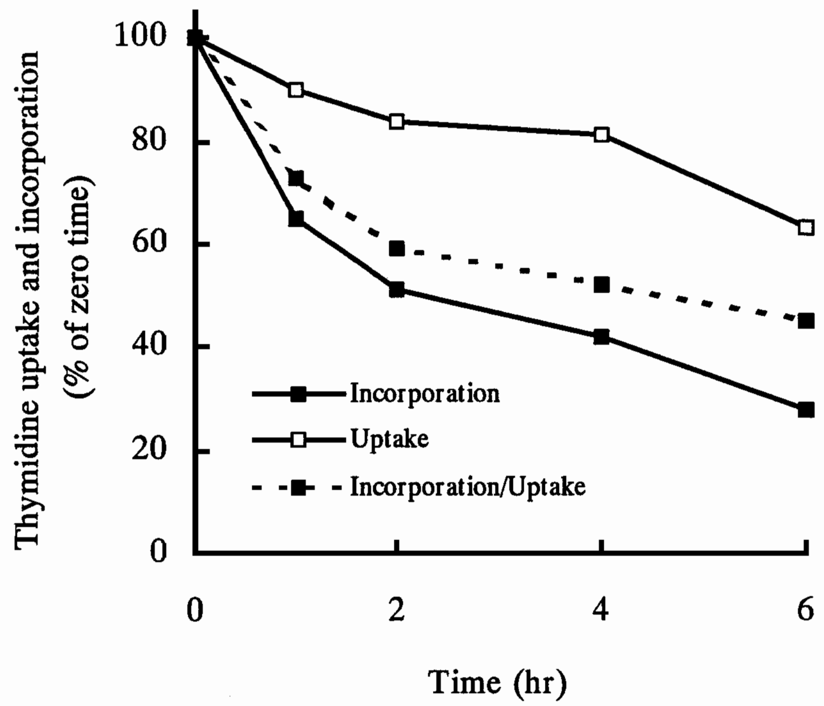


Fig. 6.2 Effect of imazosulfuron (10 ppb) on [^{3}H]thymidine incorporation into DNA in 7-day-old CYPSE root tips

The zero-time values are 2.81 kBq / 100 mg fresh weight of root tips for incorporation in DNA.

acids (Table 6.2). An exogenous supply of these amino acids at a concentration of 100 ppm or above reversed the amount of thymidine incorporation into DNA synthesis more than that of the control.

The recovery of thymidine incorporation rates began within 2 hr after addition of the three amino acids to roots that had been pretreated for 24 hr with imazosulfuron, and reached almost the same degree as the control within 6 hr (Fig. 6.3).

Effect of imazosulfuron on soluble protein levels

The level of soluble protein in root tips treated with 3 ppb and 10 ppb of imazosulfuron decreased to respectively 85% and 79% of that of control 8 days after treatment, but recovered to 94% and 90% during the following 11 days (Fig. 6.4). While those treated with the herbicide at 30 ppb and 100 ppb continued to decrease for 11 days after the treatment. The level treated with 100 ppb of the herbicide reached 47% of the control in the following 11 days.

Effect of imazosulfuron on free amino acid levels

The level of free amino acids measured in root tips treated with imazosulfuron increased with higher concentration of the herbicide (Fig. 6.5). The level treated with 3 ppb, 10 ppb and 30 ppb of the herbicide was 175%, 225% and 343% of the control 4, 4 and 8 days after treatment, respectively, and thereafter slightly decreased, while amino acids treated with 100 ppb of the herbicide increased to 354% of the control in the following 11 days without subsequent decrease.

Table 6.2 Effects of valine, isoleucine and leucine on the inhibitory action of imazosulfuron (10 ppb) on DNA synthesis in 7-day-old CYPSE root tips

Amino acid	Concentration (ppm)	$[^3H]$Thymidine incorporation (Bq*)	(% of Control)
Control	-	230	-
Val+Ile+Leu	0	127	55
Val+Ile+Leu	30	195	85
Val+Ile+Leu	100	245	107
Val+Ile+Leu	300	263	114

*, Numbers followed by the same letter are not significantly different at the 0.05 level by Tukey's Multiple Range Test.
Bq, Bq / 100 mg fresh weight of root tips

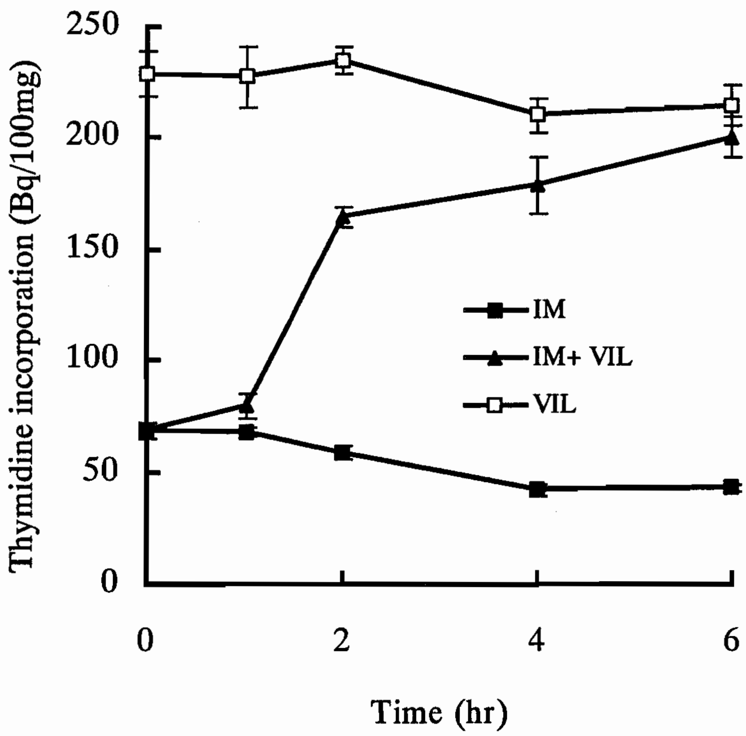


Fig. 6.3 Effects of valine, isoleucine and leucine on imazosulfuron inhibition of [^{3}H]thymidine incorporation into DNA in 7-day-old CYPSE root tips

Vertical bars represent the standard deviation.

IM, imazosulfuron (10 ppb)

VIL, valine, isoleucine and leucine (300 ppm)

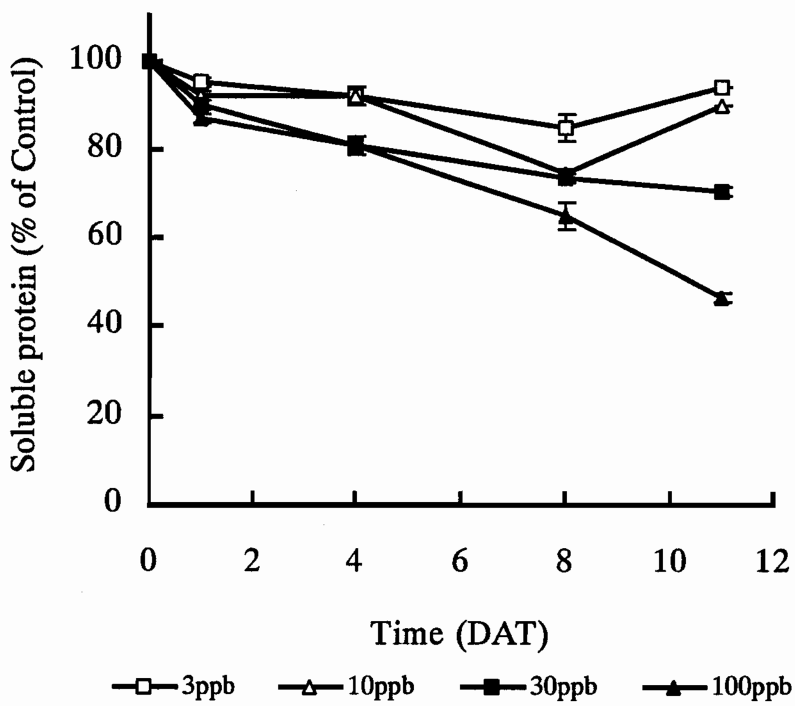


Fig. 6.4 Effect of imazosulfuron on soluble protein level in 7-day-old CYPSE root tips

The control values (±standard errors of the mean) of 1, 4, 8 and 11 days after treatment are 3.9±0.02, 3.7 ± 0.01, 4.0 ± 0.03 mg/g fresh weight, respectively. Vertical bars represent standard errors of means.

DAT, days after treatment

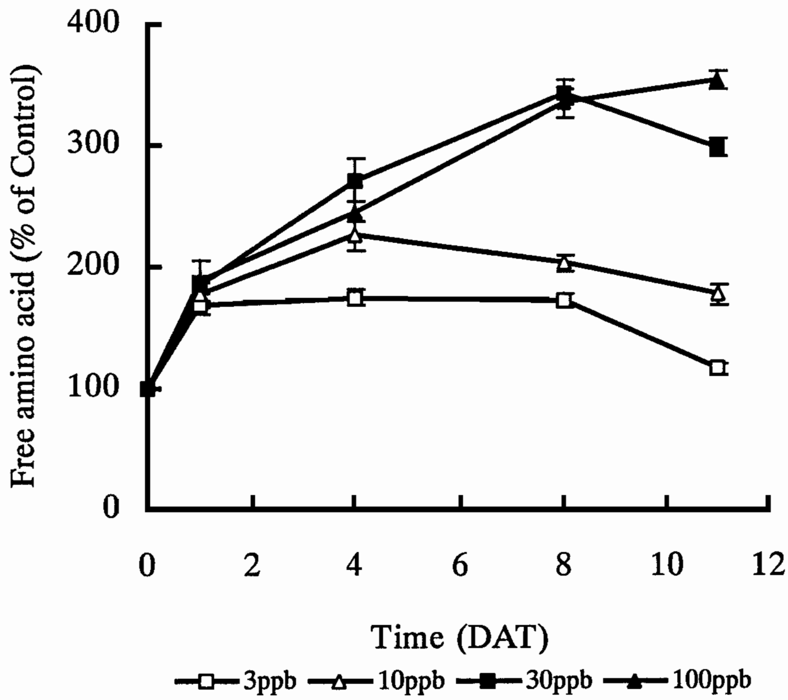


Fig. 6.5 Effect of imazosulfuron on free amino acid level in 7-day-old CYPSE root tips

The control values (±standard errors of the mean) of 1, 4, 8 and 11 days after treatment are 6.1±0.29, 8.4±0.24, 6.4±0.20 and 6.9±0.10 mM leucine equivalent/g fresh weight, respectively. Vertical bars represent standard errors of means.

DAT, days after treatment

DISCUSSION

Branched-chain amino acids alleviated growth inhibition in CYPSE, which is sensitive to imazosulfuron, as their concentrations and treatment duration increased (Fig. 6.1). These amino acids also reversed growth inhibition in rice plants which is tolerant to the herbicide (Chapter 5). This result and information indicates that the inhibitory action of imazosulfuron is alleviated by the addition of these amino acids irrespective of the sensitivity of the plant to the herbicide. Imazosulfuron inhibited ALS in both plants (Chapter 5). I also suggest that inhibition of ALS by imazosulfuron caused the depletion of the branched-chain amino acids, valine, isoleucine and leucine.

The treatment with imazosulfuron inhibited the proportion of thymidine incorporation into DNA within a few hours after treatment (Table 6.1, Fig. 6.2). Inhibited thymidine incorporation, however, was largely alleviated within 2 hr with exogenouly adding of branched-chain amino acids (Fig. 6.3). A concentration of 10 ppb (approximately 24 nM) of imazosulfuron, which is nearly identical to I_{50} for ALS, suppressed DNA synthesis to 51%. These results suggest that the primary action of imazosulfuron may involve DNA synthesis, although the concentration of 100 ppb did not increase the inhibition of DNA synthesis. Moreover, the slope of inhibition curve during 4 hr and 6 hr after treatment was more gradual than that observed during the first 2 hr, and this was partly due to the uptake inhibition. The action of chlorsulfuron on plant DNA synthesis appeared to be indirect [35], which has been suggested to be mediated by a lack of induction of ribonucleoside diphosphate reductase following a depletion of the branched-chain amino acid pool [34]. I therefore suggest that imazosulfuron inhibits DNA synthesis indirectly by lowering valine, isoleucine and leucine levels leading to reduced DNA precursor synthesis as do other ALS inhibitors.

Prompt alleviation of inhibited thymidine incorporation by branched-chain amino acids suggests that depletion of these amino acids induced by imazosulfuron causes the inhibition of thymidine incorporation.

The level of soluble protein decreased with time and concentration of imazosulfuron treatment, while increasing free amino acid levels were measured with higher concentration of the herbicide. There was no detectable decrease in the rate of radiolabeled leucine incorporated into protein by briefer imazosulfuron application (Table 6.1). These results suggest that imazosulfuron does not directly inhibit the protein synthesis. Protein hydrolysis induced by sulfonylurea herbicides caused an increase in amino acids [73, 74]. A high correlation between the pool sizes of valine and leucine and the amount of growth inhibition caused by imazaquin treatment suggested that growth inhibition was the result of depletion of these two amino acids. Therefore, depletion of valine, isoleucine induced by imazosulfuron treatment could lead to protein turnover and an increase in the level of amino acids in a longer period.

SUMMARY

Various physiological responses were studied in CYPSE following treatment with imazosulfuron. RNA synthesis measured by [^{3}H]uridine incorporation and DNA synthesis measured by [^{3}H]thymidine incorporation were inhibited to 82% and 51%, respectively, 4 hr after treatment of 10 ppb of imazosulfuron, while protein synthesis measured by [^{3}H]leucine incorporation was not inhibited. Inhibition of DNA synthesis began within 1 hr after treatment and reached 28% of the zero time value of radioactivity after 6 hr.

The amount of reversal from the reduction in plant height induced by 3 ppb or more of imazosulfuron increased further when valine, isoleucine and leucine were

added at higher concentrations. Three amino acids at 100 ppm or above alleviated DNA synthesis reduced by 10 ppb of imazosulfuron following 4 hr more than the control. Supplement of amino acids at 300 ppm greatly alleviated the inhibition of DNA synthesis within 2 hr and did the same to the control to almost the same degree 6 hr following pretreatment of imazosulfuron for 24 hr.

The level of the soluble proteins decreased to 47% of the control 11 days after treatment with a concentration of 100 ppb of imazosulfuron, while the free amino acid level increased to 354% of the control.

Starvation of valine, isoleucine and leucine induced by imazosulfuron is thought to cause the inhibition of DNA synthesis in a short period of treatment and lead to protein turnover and increase in the level of amino acids over a longer period.

DISCUSSIONS WITH RESPECT TO THE HERBICIDAL PROPERTIES OF IMAZOSULFURON

It is important to understand the biological efficacy and selectivity of paddy herbicides as well as investigating the physiology of paddy weeds. From practical viewpoints, it is also greatly important to elucidate the factor affecting the herbicidal activity.

Imazosulfuron controls paddy weeds including annual and perennial broadleaf and sedge weeds on pre- and post-emergence application with sufficient residual activity, and has enough safety to rice plants, because relative movement of the herbicide in paddy soil is limited to within 2 cm of the soil layer under the soil surface. Post-emergence application of imazosulfuron had the stable activity on paddy weeds irrespective of flooding water depth, temperature, water leaching, planting depth and tuber size. However, water leaching and deeper planting depth reduced significantly the efficacy with pre-emergence application. The scheme of reduction of efficacy induced by water leaching with pre-emergence application is proposed as shown in Fig. 7.1. Supposedly, water leaching intensified vertical movement of imazosulfuron is thought to reduce the amount of the herbicide on the soil surface where the seed was sawn. Thus, this reduction of imazosulfuron may result in decreasing absorption of the herbicide for seed and root. Moreover, imazosulfuron suppressed the initial growth of paddy weeds until either coleoptile or 1 leaf-stage to a lesser degree than in the later stages of growth. Namely, growth of plants ceases prior to 1-leaf stage with pre-emergence application of the herbicide. Similar suppression was shown in the pre-emergence application on a moderately susceptible weed, ECHOR and in the residual activity on SAGPY and SCPJO. Judging from these results, decrease in the growth suppression of initial growth stage also may intensify the reduction of herbicidal activity by water leaching. According to the result that tolerant of rice plant to imazosulfuron does not depend on the sensitivity of ALS activity irrespective the

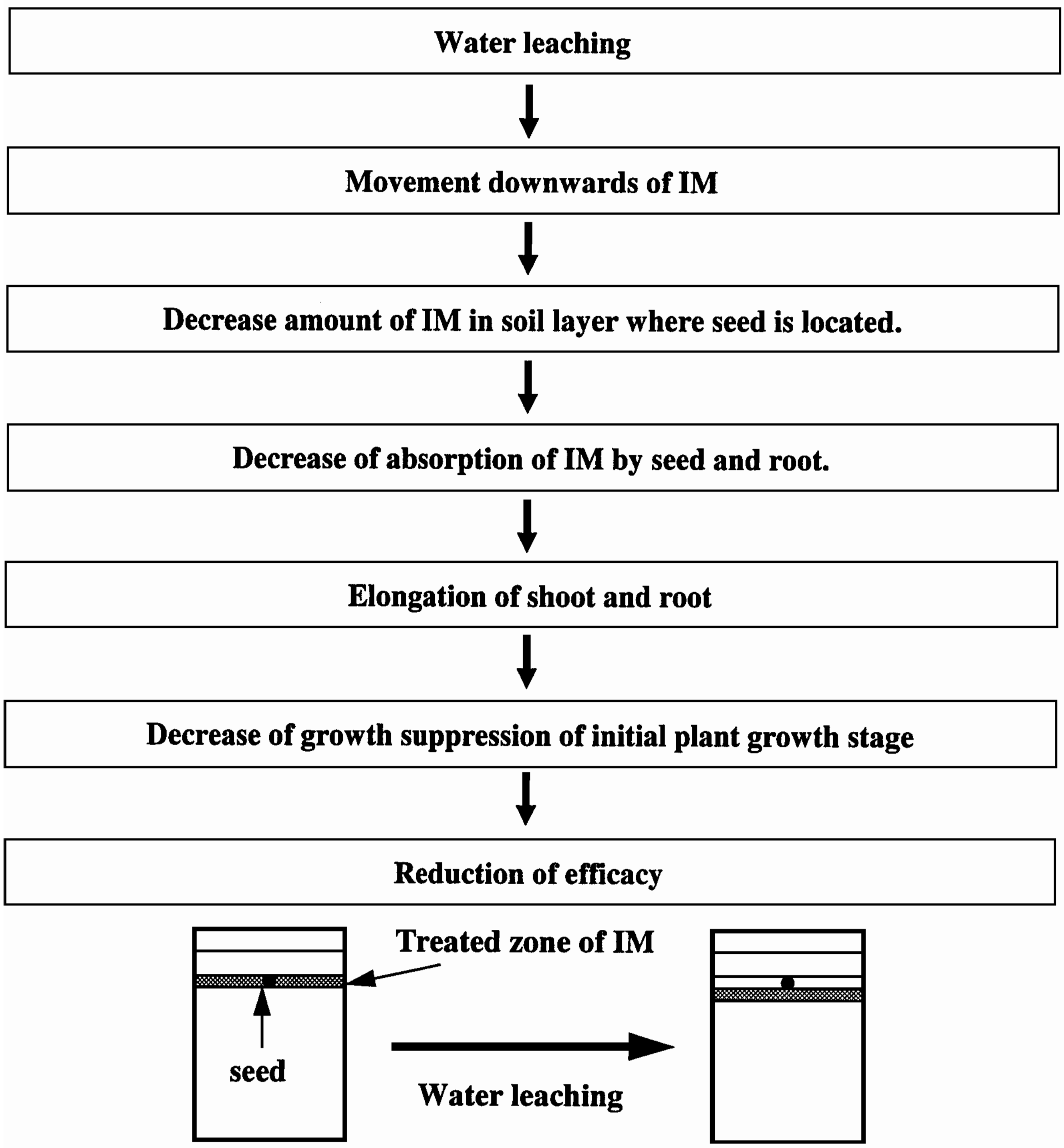


Fig. 7.1 Proposed scheme of reduction of efficacy induced by water leaching with pre-emergence application
IM, imazosulfuron

difference in plant age, these suppressions do not seems to relate ALS activity.

With non-leaching condition, enough amount of imazosulfuron to completely inhibit the growth of the paddy weeds may not move downward in the soil layer whichthe seeds are placed. This seems to relate to limited movement of imazosulfuron in the soil. Besides the length of mesocotyl or basal shoot and distribution of the growing point in paddy weeds may affect the herbicidal activity. The length of mesocotyl depends on the plant species and the depth the seed was placed. On the basis of the above-mentioned results, proposed scheme is shown in Fig. 7.2. Consequently, more ecological understanding of paddy weeds is needed to have relation with herbicidal property. Factors affecting movement of imazosulfuron may be also important. One of them seems to be soil property related to adsorption and water solubility of the herbicide. Further studies are needed to elucidate these relationships.

The scheme of enhancement factors of phytotoxicity is also proposed as shown in Fig. 7.3. Shallow planting of rice plants may be an important factor caused the phytotoxicity by imazosulfuron. The underground parts, roots and basal leaf sheath, of the shallowly planted easily come into contact with the herbicide on the soil surface because of lack of protection by the soil. Imazosulfuron was metabolized in rice plants faster than in CYPSE [51]. However, too much of the herbicide does not seem to be immediately metabolized in the plants. Rice plants transplanted at a 2 cm depth or more did not suffer a growth inhibition induced by water leaching , early application timing and higher temperature. On the other hand, these factors intensified the phytotoxicity of shallowly transplanted rice plants treated with imazosulfuron. These factors are thought to be intensifiers as described below. Water leaching enhances the downward movement of imazosulfuron to root zone of the plants. Early application of the herbicide inhibits the growth of rice plants prior to rooting in the soil. Higher temperature increases the absorption of the herbicide by root of rice plants. Water leaching, early application timing and higher temperature did not suggest to be

Deeper planting → **Mesocotyl does not reach to soil surface.** ; **Growing point is located below soil surface.**

Non-leaching → **Movement downwards of IM is limited.**

↓

Decrease amount of IM in soil layer where growing point and root are located.

↓

Decrease of absorption of IM by seed and root.

↓

Elongation of shoot and root

↓

Decrease of absorption of IM by seed and root

↓

Reduction of efficacy

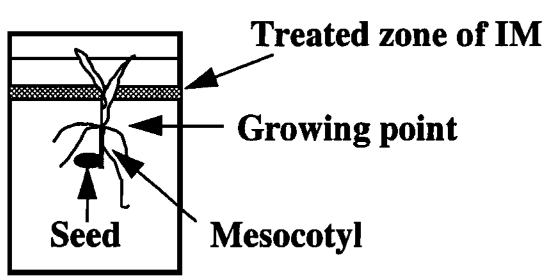


Fig. 7.2 Proposed scheme of reduction of efficacy induced by deep planting with pre-emergence application
IM, imazosulfuron

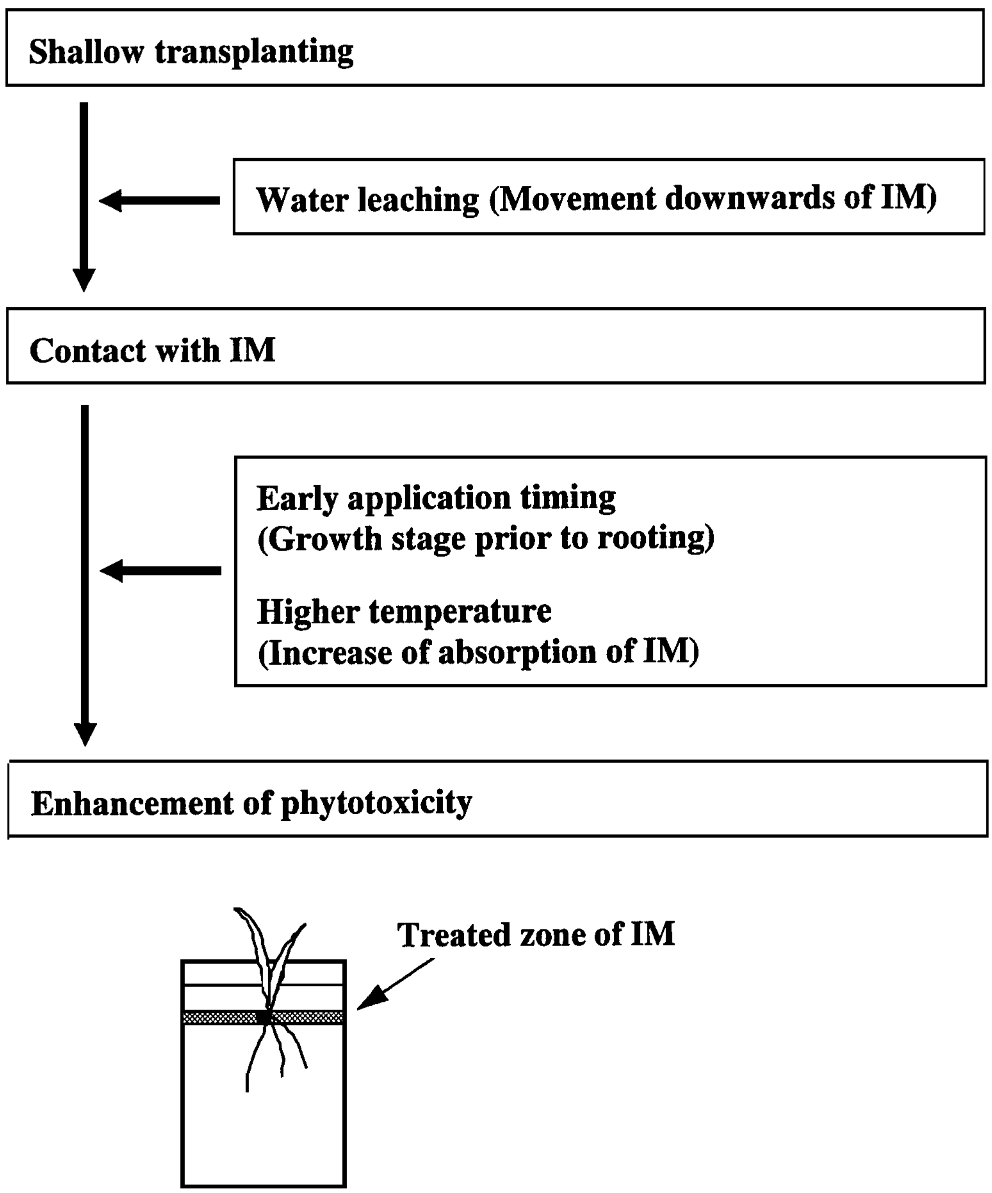


Fig. 7.3 Proposed scheme of enhancement of phytotoxicity induced by shallow transplanting
IM, imazosulfuron

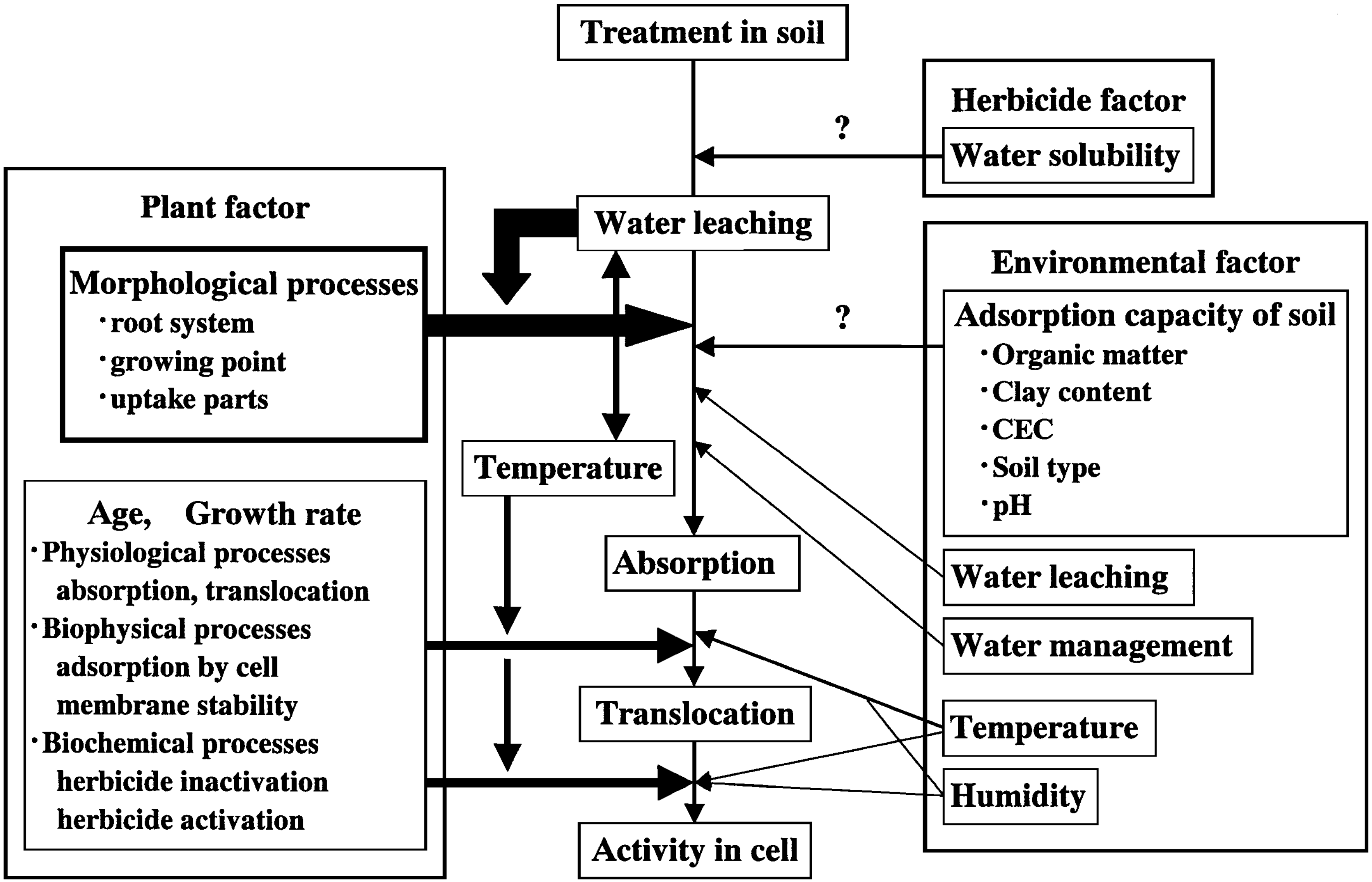


Fig. 7.4 Proposed scheme of mechanisms of factors affecting imazosulfuron activity

important factors for practical use, but be important in relation to the shallow transplanting.

According to these results, the scheme of mechanisms of factors affecting imazosulfuron activity is proposed as shown in Fig. 7.4. Morphological processes (root zone, growing point, uptake parts) are the most important. Water leaching affects the morphological processes, and varies the weed control and crop injury. Temperature also influences the plant growth in relation to the variation with water leaching. The relationship among imazosulfuron activity, water solubility and adsorption capacity of soil has not been clear. Further studies are needed to investigate the relation.

In consideration of the alleviation effect of branched-chain amino acids and direct inhibition of ALS, imazosulfuron is thought to inhibit ALS from the plants in a same manner as the other sulfonylureas on the enzyme.

Contrast between I_{50} values for the intact plants and for ALS indicates that the selectivity of imazosulfuron does not seem to be due to the difference in the enzyme level reaction with ALS. The morphology of a plant in root systems, location of growing points and uptake parts can determine the selectivity between rice plants and paddy weeds. Most paddy weeds can be easily controlled because of shallow root systems, whereas rice plants have deep, extensive root systems. Such deep root systems may decrease crop injury from imazosulfuron that remains near the soil surface through depth-protection. In CYPSE imazosulfuron was mostly absorbed by root and partly by shoot, while in rice plants by both root and basal leaf sheaths. Shoots parts in broadleaf weeds that are important for herbicide uptake are the hypocotyl, epicotyl and cotyledon, whereas the most important shoot parts in grasses are the coleoptile and mesocotyl [17]. This factor may be based on the selectivity between rice plants and broadleaf weeds. These results support that the difference in the metabolic rate of imazosulfuron between rice plants and CYPSE may cause selectivity between them like other sulfonylureas.

According to the results of the inhibition properties, it is suggested that imazosulfuron does not overlap with the binding site on ALS where pyruvate or TPP have overlapped but inhibits the enzyme activity by binding the complexes of the enzyme, cofactor and substrate. Moreover, imazosulfuron is thought to be a slow-binding inhibitor. The maximum rate constant for the (time-dependent) transition between the initial and the final, steady-state inhibition by imazosulfuron was nearly identical to those of other ALS-inhibiting herbicide. This result may support the hypothesis of Hawkes [40] that the apparent slow phase of ALS inhibition by ALS-inhibiting herbicides is due to slow irreversible inactivation of the enzyme rather than isomerization of the enzyme-inhibitor complex to a more tightly bound form. These slow-binding properties are assumed to be one of the major factors for the extremely high herbicidal activity of imazosulfuron as well as other sulfonylureas.

The incomplete inhibition of DNA synthesis by imazosulfuron and the alleviation effect of valine, isoleucine and leucine indicate the imazosulfuron may indirectly inhibit the DNA synthesis by lowering the level of these amino acids. Inhibition of the synthesis of DNA seems to be important secondary events that follow from the inhibition of branched-chain amino acid biosynthesis.

Results that no direct inhibition of the protein synthesis in early period after treatment and soluble protein decrease in later period afterwards indicates that imazosulfuron does not inhibit the protein synthesis directly. The soluble protein decrease seems to be due to protein hydrolysis to amino acids by protein turnover. The turnover rate is suggested to respond to the enhancement of amino acids starvation.

I suggest that starvation of branched-chain amino acids induced by imazosulfuron cause the inhibition of DNA synthesis in a short period of treatment, and lead to protein turnover and an increase in the level of amino acids in a longer period.

According to the above-mentioned results, the scheme of physiological responses with respect to the mode of imazosulfuron action is proposed as shown in Fig. 7.4.

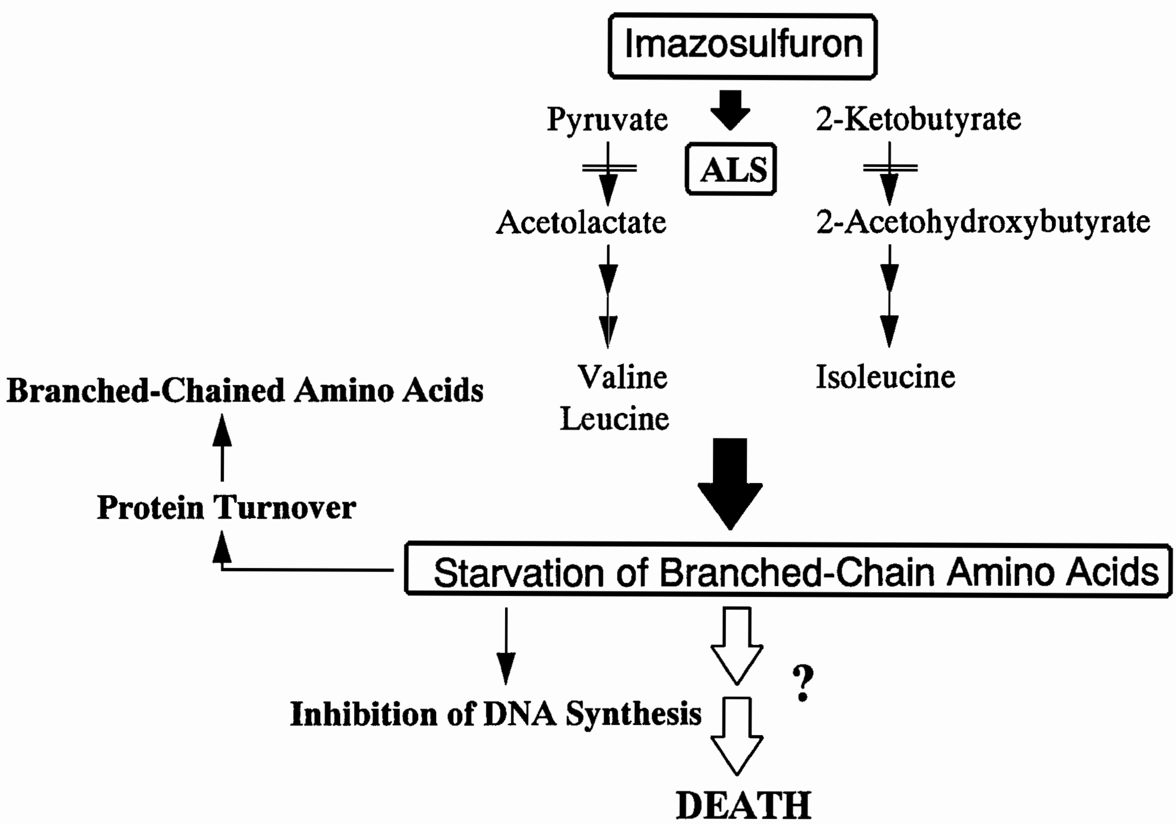


Fig. 7.4 Proposed scheme of the physiological responses with respect to the mode of imazosulfuron action
ALS, acetolactate synthase

The connection between inhibition of ALS and general growth inhibition lead to death has not been clear. Further studies are needed to investigate the relationship of them.

Since the introduction of sulfonylureas including imazosulfuron, the technology in use of rice herbicide in Japan has been advanced through the development of application methods, low application rate and formulation technology. Especially, labor time for weeding in paddy fields has been saved owing to the simplification of application methods, including reduction of the amount of granule formulation, suspension concentrate and throw-in formulations, as well as reduction of the application frequency by the development of one-shot herbicides. Imazosulfuron, which is an excellent rice herbicide having the most appropriate balance between herbicidal activity and crop selectivity, could be applicable in combination with grass herbicides simultaneously when rice plants were transplanted automatically by transplanter without restrict on the use dosage and use area. This application method has promoted the development of new herbicide application and formulation.

Considering these circumstances, there is a continuous need for updating herbicide technology because of changes and developments in agricultural practices. These information obtained here will contribute to the weed control in rice cultivation system for development of labor-saving and lower cost technology, and the weed science for environmental compatibility and assessment in agricultural ecology system.

SUMMARY

Imazosulfuron, which is a sulfonylurea herbicide synthesized and developed by Takeda Chemical Industries, Ltd. for use in paddy rice, was studied for biological activity, factors affecting biological activity, selectivity and mode of action.

Imazosulfuron controlled 12 paddy weeds including annual and perennial broadleaf and sedge weeds on pre- and post-emergence application of 90 g a.i./ha with sufficient residual activity for 42 days. Bioassay method showed that relative movement of the herbicide in paddy soil was limited to within 2 cm of the soil layer under the soil surface. Herbicidal activity was roughly proportional to the exposure period of between 1 and 7 days with different sensitivity of paddy weeds. Post-emergence application of the herbicide results in the stable activity on a paddy weed irrespective of flooding water depth, temperature, water leaching, planting depth and tuber size. Water leaching and planting depth significantly reduced the herbicidal activity with pre-emergence application. Water leaching and planting depth with pre-emergence treatment suggest to be important factors influencing the herbicidal efficacy of imazosulfuron.

Selectivity between paddy weeds and rice plants on imazosulfuron was investigated. I_{50} value of *Cyperus serotinus* (CYPSE), *Sagittaria pygmaea* (SAGPY), *Scirpus juncoides* var. *ohwianus* (SCPJO) and *Echinochloa orizicola* (ECHOR) was approximately from 500 to 2200 times lower than that of rice plants treated 7 days after transplanting. This result suggests that imazosulfuron has sufficient safety to rice plants (*Oryza sativa* cv. Nipponbare, ORYSA) at a dosage of 90 g a.i./ha for practical use.

Absorption site of imazosulfuron in CYPSE and rice plants was estimated by using three application methods. CYPSE treated with soil application of the herbicide suffered the most growth suppression, that with soil incorporation application the next, that with flooding water application the least. On the other hand, the growth inhibition

of rice plants with soil application was similar to that with soil incorporation application, and both inhibitions were higher than that with flooding water application. In CYPSE imazosulfuron seems to be mostly absorbed by root and partly by shoot, while in rice plants by both root and basal leaf sheaths.

The growth reduction of rice plants, when transplanted at a depth of 2 cm or more, was rarely indicated by treatment of imazosulfuron irrespective of water leaching and differences in application timing, flooding water depth and temperature. Shallowly transplanted rice, however, suffered significant growth reduction by treatment of imazosulfuron. Transplanting depth seems to be the most important factor affecting the phytotoxicity on rice plants by imazosulfuron. The growth reduction was intensified by water leaching, early application timing and higher temperature after application when rice plants were transplanted shallowly. These suggest to be also very important factors influencing the phytotoxicity on the plants by the herbicide in shallowly transplanted depth.

Addition of valine, isoleucine and leucine reversed growth inhibition of rice plants induced with imazosulfuron. I_{50} for acetolactate synthase from both sensitive and tolerant plants was 14 to 58 nM. These results strongly support that imazosulfuron acts on plant tissue by directly inhibiting the enzyme ALS. Taking the contrast between I_{50} values for the intact plants and for ALS into consideration, the selectivity of imazosulfuron does not seem to be due to the difference in the enzyme level reaction with ALS. The ALS activity which chloroplast development of rice plants enhances did not overcome the inhibition induced by imazosulfuron. The insensitivity of rice plants to imazosulfuron is probably also not due to the enhancement of ALS activity relative to age and chloroplast development.

Imazosulfuron inhibited ALS activity in the noncompetitive with respect to pyruvate and uncompetitive with respect to thiamine pyrophosphate (TPP). Inhibition of ALS activity by the continuous presence of imazosulfuron was time dependent and biphasic. Therefore, imazosulfuron does not suggest to overlap with the binding site on ALS where pyruvate or TPP have overlapped but to inhibit the enzyme activity by binding the complexes of the enzyme, cofactor and substrate, and to be a slow-binding inhibitor.

Various physiological responses following treatment with imazosulfuron were investigated using CYPSE. Imazosulfuron inhibited the proportion of thymidine incorporation into DNA within a few hours after treatment and inhibited to 51% of the control. This inhibition was largely alleviated within 2 hr with exogenously adding of branched-chain amino acids. The level of soluble protein decreased with time and concentration of imazosulfuron treatment, while increasing free amino acid levels were measured with higher concentration of the herbicide 11 days after treatment of the herbicide. Starvation of valine, isoleucine and leucine induced by imazosulfuron seem to cause the inhibition of DNA synthesis in a short period of treatment and lead to protein turnover and an increase in the level of amino acids in a longer period.

The technology in use of rice herbicide in Japan has been advanced through the development of new active ingredients, application methods, low application rate and formulation technology. The rational herbicide use technology will be more promoted by using information obtained here. These advancement will contribute to the weed control in rice cultivation system for development of labor-saving and lower cost technology, and the weed science for environmental compatibility and assessment in agricultural ecology system.

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