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Ebrima Madi Kunjo for the degree of Master of Science in Crop Science
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Title: Emergence and Growth of Nine Accessions of Diclofop-Resistant
Italian Ryegrass (*Lolium multiflorum* L.) and Multiple Resistance to
Other Herbicides.

Abstract approved: _____
Myron Shenk

Italian ryegrass (*Lolium multiflorum* L.) is a serious annual grass
weed which occurs in small grain fields, pastures, roadsides, gardens
and wastelands in the Willamette Valley of western Oregon. Diclofop
(($\pm$)-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoic acid) (Hoelon) has
been successfully used for controlling this species and wild oats (*Avena
fatua* L.) in cereal grains and other crops since 1977. Failures in
control of ryegrass began to appear after several years of use, and
resistance of this species to Hoelon in Oregon was confirmed in 1987
(Ritter, 1989).

Field and greenhouse experiments were conducted from October, 1991
to May, 1992, to study the emergence and growth of nine Hoelon-resistant
ryegrass accessions, and to determine their resistance to other
herbicides with similar or dissimilar modes of action; including
pronamide (3,5-dichloro-N-(1 dimethyl-2-propynyl) benzamide), UBIC4243
(common name not yet released), triallate (S-(2,3,3-trichloro-2-
propenyl) bis ((1-methylethyl) carbamothioate), trifluralin (2,6-

dinitro-N,N-dipropyl-4-(trifluoromethyl) benzenamine N-dipropyl-p-tolyidine), diuron (N'-(3,4-dichlorophenyl)-N,N-dimethylurea) and metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one).

Control of all accessions was 100% three weeks after applying 1.1 kg/ha of pronamide preemergence. Control with 0.8 kg/ha of trifluralin preplant-incorporated was 95% for all accessions 3 weeks after treatment (WAT). This pattern of control was the same in field and greenhouse trials.

Postemergence application of 1.8 kg/ha of diuron controlled 100% of eight accessions 3 WAT. Accessions 4 and 8 were highly resistant to 1.8 kg/ha of diuron in both field and greenhouse, with control of only 3 and 10%, respectively. This expression of tolerance or resistance to diuron persisted for the 12 week duration of the field experiment and the 6 week duration of the greenhouse experiment.

Control of accession 10 (commercial population) was 100% with 1.1 kg/ha of diclofop.

Biomass weight for the diuron-resistant accessions was comparable to that of respective untreated checks. Biomass weights of accessions that were susceptible to all herbicides, were reduced an average of 80% as compared to their respective untreated checks. Emergence rate of the diuron resistant accession was slower than susceptible accessions in the greenhouse

This study suggests that development of resistance by Italian ryegrass to diclofop-methyl appears to be common in western Oregon. The phenomenon also appears to be independent (location-specific) depending

on factors such as type of cropping system, and duration of use of a given herbicide (i.e. selection pressure).

Emergence and Growth of Nine Accessions of
Diclofop-Resistant Italian Ryegrass (*Lolium multiflorum* L.)
and Multiple Resistance to Other Herbicides

by

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Last, but not the least, to my daughters Fatoumatta and Adama, who went through another period of both maternal and paternal absence with so much understanding and love.

Finally, to the Great Almighty Allah whose guidance inspired me to persevere whenever conditions seemed difficult, and who also showed light each time darkness came.

DEDICATION

I would like to dedicate this thesis to my wife, Bintou, and my children Lamin, Fatoumatta and Adama. Completion of this work would not have been possible without Bintou's love, support, and encouragement. My children's concern to see me succeed in life has brought a new dimension to my life and happiness that I wouldn't trade for anything.

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LIST OF ABBREVIATIONS

kg ai/ha = kilograms active ingredient per hectare

ANOVA = analysis of variance

WAT = weeks after treatment

PRE = pre-emergence

PPI = pre-plant emergence

OM = organic matter

R = resistant

S = susceptible

cm = centimeter

m^2 = meter square

CRD = completely randomized design

Con G/L = concentration, grams per liter

L/ha = liters per hectare

DPE = directed postemergence

EMERGENCE AND GROWTH OF NINE ACCESSIONS OF
DICLOFOP-RESISTANT ITALIAN RYEGRASS (*Lolium multiflorum* L.)
AND MULTIPLE RESISTANCE TO OTHER HERBICIDES

INTRODUCTION

Weeds are often the primary limiting factor in crop production
systems in many regions of the world. Herbicides are used to control
weeds in many countries around the world. However, as in the case of
chemical control of insects, where more than 500 cases of resistance
have now been recorded, weeds are beginning to develop resistance to
herbicides. By early 1990, more than 100 cases of weed resistance were
reported on a world scale (LeBaron et al., 1990). In recent years,
Italian ryegrass (*Lolium multiflorum*) has developed resistance to the
diphenyl-ether herbicide, diclofop-methyl (Hoelon), on at least three
continents (Heap et al., 1986). This is especially important to Oregon
farmers since Italian and perennial ryegrass are produced as seed crops
on approximately 174,000 acres (70,000 ha). Sixty percent of this
acreage is Italian ryegrass. Conversely, Italian ryegrass is considered
a serious weed in many crops in Oregon. It is well adapted to the
climate, and its trait of outcrossing provides this species with the
potential to continually adapt to a wide range of climatic and agronomic
conditions.

A serious problem associated with the development of resistance is
the development of cross-resistance. In this case, an organism develops
increased resistance to a given pesticide as a result of selection
pressure imposed by another pesticide. Cross resistance is more likely
to occur with pesticides of similar mode or site of action, as is the

case with the S-triazine group (Gressel, 1979). Biotypes of *Chenopodium album* L., *Senecio vulgaris* L. and *Amaranthus retroflexus* L., which were resistant to simazine, were cross-resistant to most S-triazine herbicides (Radosevich and Appleby, 1973; Bandeen and McLaren, 1976; Bradshaw and McNeilly, 1981). However, cross-resistance between pesticides of unlike modes of action also exists (Heap and Knight, 1986). In some cases, insects have developed resistance to insecticides from three or more different insecticide groups all with similar or dissimilar mode or site of action. This reduces the chemical options available for controlling such species.

It is a common occurrence in biology that whenever a particular trait is selected for, the selected individuals are often less fit (less competitive). It has been predicted that once selected, herbicide-resistant weeds would also be less fit than their susceptible counterparts, which would permit their control with other methods without serious consequences (LeBaron et al., 1982). However, should the resistant species be equally competitive, or even more fit, they could present serious management problems.

Italian ryegrass is a serious weed in wheat, barley, peas and other grass and legume seed fields in Oregon. Diclofop has been used for controlling Italian ryegrass and wild oats in cereal grains and other crops. However, in recent years, reports of Hoelon failure began to appear. It is now an accepted fact that ryegrass is resistant to Hoelon in many fields throughout the Willamette Valley of Western Oregon. Holliday et al. (1977) suggested that development of resistance to herbicides invariably occurs in environments which particularly favored rapid evolution such as when a herbicide treatment is maintained

over many consecutive years of cropping a particular field. Hoelon has been used for almost 15 years in some fields in the Willamette Valley.

Because of the biological similarity of Italian ryegrass to some of the predominant upland grass weed species found in the author's home country (The Gambia), this study will be important in the search for appropriate management practices of these weed species. In addition, herbicides from chemical groups similar to Hoelon, and others, are being used in The Gambia against these species; some for more than a decade. Fortunately, at this time, there are no reports of resistance developing in these species in The Gambia toward the herbicides being used to control them.

The objectives of this study include the following:

1. To verify the existence of Hoelon-resistant Italian ryegrass in different areas in Western Oregon.

2. To determine if Hoelon-resistant biotypes from different locations in Western Oregon are resistant to other herbicides with similar and dissimilar modes of action (cross-resistance or multiple-resistance).

3. To study certain biological characteristics of resistant biotypes which might be utilized to help manage this weed under field conditions, such as emergence and seedling vigor (speed of emergence and seedling growth evaluation).

LITERATURE REVIEW

2.1 Botanical Characteristics of Italian Ryegrass as a Weed

Italian ryegrass (*Lolium multiflorum* L.) is a short-lived grass with 30-80 cm tall stems which are usually rough below the spike. This plant species occurs in small grain fields, pastures (especially those planted to orchardgrass and tall fescue), roadsides, gardens, and wastelands. It is a native of Europe. In the Willamette Valley, Italian ryegrass has been identified as one of the most serious weed species in wheat, barley and other grass species and legume seed fields (Lorenzi et al., 1987).

Diclofop-methyl (Hoelon) has been successfully used for controlling this weed and wild oats in cereal grains and other crops since 1977. Failures to control ryegrass began to appear after several years of use and resistance to Hoelon in Oregon was confirmed in 1987 (Ritter, 1989).

2.2 Definition and Recognition of Resistance

According to LeBaron and Gressel (1982), resistance is a decreased response of a population of animal or plant species to a pesticide or control agent following one or more applications. The term herbicide resistance is applied to a weed population within a species that is normally susceptible to a particular herbicide but with repeated exposure over time, it is no longer susceptible to normal rates of the same herbicide. This definition suggests that repeated applications of a herbicide are needed. The development of diclofop- (Hoelon) resistant Italian ryegrass is a good example of this phenomenon.

The development of resistance has great significance to growers and the crop-protection industry whenever increased rates of herbicides are necessary to provide control of resistant populations.

Early recognition of resistant populations in the field is likely to be difficult for growers since failure to control weeds may occur for various reasons. Holliday et al. (1976) noted that it would be difficult to determine whether failure of control was attributable to use of an inferior grade or formulation of a herbicide, incorrect dosage, faulty application, unsatisfactory environmental conditions, or to a real change in the susceptibility of a weed population. With any of these possibilities, surviving individuals could appear to be resistant.

2.3 Occurrence of Herbicide Resistance in Weeds

Evolution of insecticide resistance was the inevitable result of widespread use of insecticides. A parallel increase in the use of herbicides led to predictions that evolution of genetically-resistant weed populations would occur (Abel, 1954 Harper, 1956).

The first recorded incidence of herbicide resistance in weeds involved a triazine herbicide and groundsel (*Senecio vulgaris*), a broadleaf weed in a stand of conifer trees. This occurred in 1968 in the state of Washington, U.S.A. (LeBaron and Gressel, 1982). During the 1970's and 1980's, notably in North America and Western Europe, a number of new species of weeds developing resistance to triazine and other herbicides has increased relatively steady (Putwain, 1982). Dodge (1989), in personal communication with LeBaron, reported that on a worldwide scale, 49 species of 33 genera have become resistant to

triazine herbicides and a further 11 species have evolved resistance to other herbicides.

The occurrence of herbicide-resistant biotypes may indicate that numerous populations have independently evolved in many different locations over a period of just a few years. Hartman (1979), reported that in Hungary, *Amaranthus retroflexus* resistance to S-triazines occurred in scores of locations and that 75% of the maize growing area has become infested by the resistant biotype. In the USA, evolution of resistance to S-triazines in *Amaranthus hybridus* first occurred in Maryland in 1972, but between 1976 and 1982, there were numerous reports of resistant populations occurring in several other states (LeBaron and Gressel, 1982). Putwain (1982) noted that some species appear to have a tendency to evolve resistance to triazines possibly because genetic variations for resistance is widely dispersed through many locations. He further contended that it was unlikely that chance caused *Poa annua* (annual bluegrass) to have evolved resistance to triazines in six European countries and the USA, as well as to have evolved resistance to paraquat (1,1'-dimethyl-4-4'-bipyridinium ion) in the U.K.

Several researchers have noted resistance to herbicides other than the triazines, paraquat being one of the most frequently detected. In Japan, paraquat-resistant biotypes of *Erigeron philadelphicus* evolved in mulberry plantations (Watanabe et al., 1982). Powels (1986) noted that in Australia, a population of *Hordeum glaucum* developed resistance to paraquat after 15 years of repeated use of this herbicide. Another occurrence of the development of herbicide resistance includes populations of *Eleusine indica* to trifluralin. Mudge et al. (1984) observed that this trifluralin resistance appeared in several locations

in South Carolina after repeated use of this herbicide. Another example of herbicide resistance in weeds is that of rigid ryegrass (*Lolium rigidum*) to diclofop-methyl in Australia, reported in 15 widely scattered locations (Heap et al., 1982). From these recorded examples, it has become clear that the feature most common in the development of herbicide resistance is recurrent (usually annual) use of a herbicide over several years in monoculture cropping systems or perennial plantation crops.

Resistance of Italian ryegrass (*Lolium multiflorum*) to diclofop was first reported in the state of Oregon in 1987 (Ritter, 1989). In general, most of the cases of intraspecific variation in sensitivity to herbicides other than triazines involve biotypes that were visually recognizable prior to the introduction of the herbicide. Gressel and Segel (1978) postulated that these biotypes are ecologically "less fit" than the sensitive biotypes prior to the repeated use of the herbicides. This was observed in the studies of Radosevich (1973) with *Senecio vulgaris*.

The large increase in reported cases of weed resistance to different classes of herbicides and from different parts of the world suggests that the problem is with us to stay (LeBaron and McFarland, 1990). These authors also noted that this rapid increase of herbicide resistance endangers the usefulness of valuable old and new herbicide classes on which adequate crop protection relies.

Rubin et al. (1985) also agree that the repetitive use of high rates of the same residual herbicide (mostly triazines) along roadsides and railways, where cultivation is impractical or impossible, led to the appearance of herbicide resistant populations. However, Fuerst and

Vaughin (1990) emphasize that herbicide resistance has appeared not only when residual herbicides were heavily used, but also in cases where selection pressure is inflicted by the repeated use of herbicides like paraquat and diquat which lack soil activity.

The number and distribution of herbicide-resistant weeds has increased dramatically in the last ten years (Holt and LeBaron, 1990). In their comprehensive survey, LeBaron and McFarland (1990) documented the worldwide distribution of the phenomenon and its implication for modern agriculture. They observed that there are more than 100 weed biotypes resistant to at least 15 classes of herbicides with one or more cases in virtually all continents.

2.4 Mechanism of Herbicide Resistance

The occurrence of herbicide resistance in many weeds is often attributed to an inherited modification of the herbicide site of action. This is true for most cases of resistance to triazines, sulfonylureas and dinitroanilines. Arutzen et al. (1982) reported that triazine resistance is generally associated with point mutation of the chloroplast gene psba. This gene encodes for the photosystem II protein which serves as the herbicide binding site. In all higher plants that have been studied to date, this mutation involves a substitution of one amino acid resulting in loss of herbicide-protein affinity. This mutation also leads to an alteration of the electron transport mechanism which is expressed as a reduced energy yield for whole chain electron transport (Arutzen et al., 1982).

In considering possibilities of cross resistance, Fuerst et al. (1986), and Yaacoby et al. (1986) contended that triazine-resistant weeds often exhibit cross resistance to other herbicides having

different chemical structures such as the pyridazinones and triazinones that bind to the same site. But conversely they observed that these plants appear to remain sensitive to the substituted urea herbicide diuron, which is also a photosystem II inhibitor. Vaughn et al. (1987) reported an altered target site in a biotype of *Eleusine indica* highly resistant to bencenamine herbicides. Another case of herbicide resistance which might be (among other factors) attributable to an altered binding site is the resistance of *Stellaria media* to mecoprop, an auxin-type herbicide (Lutman and Heath, 1990). Based on stem elongation tests, Barnwell and Cobb (1989) concluded that the R biotype is far less receptive to mecoprop than the S biotype. Although not ruling out the possible involvement of differential metabolism among the biotypes, the authors suggest that the differences in mecoprop binding may form the basis for mecoprop resistance in this weed population.

Another example of herbicide resistance mechanism through the alteration of site of action is the acetyl coenzyme A carboxylase (AcCase) inhibition. This was recently discovered to be the main mechanism by which certain herbicides like diclofop-methyl affect grasses (Rendina et al., 1989).

Differential metabolism otherwise referred to as enhanced detoxification is another major mechanism of plant sensitivity to herbicides. Resistance to certain herbicides by several biotypes depends on the capacity to degrade and/or conjugate the toxic compound, hence forming less or non-toxic products, as occurs in many resistant crops. Aronwald et al. (1989) have shown that *Albutilon theophrasti* from Maryland is resistant to atrazine because of an enhanced capacity to detoxify the herbicide via a protein conjugation, as occurs in maize.

This form of enhanced metabolism was also postulated for a triazine-resistant biotype of *Echinochloa crus-galli* from France (LeBaron and McFarland, 1990). In multiple resistant annual ryegrass, no alteration of the target site nor differential uptake and translocation were observed between R and S biotypes (Powels et al., 1990). Kemp and his coworkers (1990), in a study with *Alopecurus myosuroides* (blackgrass), have clearly shown that chlorotoluron is more rapidly metabolized in the Peldon (R) blackgrass than in the S biotype (Rothamsted). Results from several studies indicate that there is possible involvement of certain enzymes such as cytochrome P_{450} mono-oxygenases in various oxidative reactions leading to detoxification of several herbicides. These studies further suggest that multiple forms of cytochrome P_{450} are present or can be induced in higher plants. Conversely, using known P_{450} inhibitors in combination with the respective herbicides, there was a significant reduction in R blackgrass growth (Kemp et al., 1988; Kemp et al., 1990; Powles et al., 1990) and less degradation of the test herbicide chlortoluron (Kemp et al., 1990). Furthermore, using a cell culture derived from the resistant Peldon blackgrass, Jones and Caseley (1989) found increased levels of P_{450} in the microsomes which was associated with increased rate of degradation of chlorotoluron by R plants.

Other documented mechanisms of herbicide resistance are sequestration and compartmentation of herbicides or their phytotoxic metabolites. Coupland et al. (1990) showed that mecoprop-resistant *Stellaria media* metabolizes the herbicide to conjugates significantly more than the susceptible plant. Sequestration of paraquat in *Erigeron philadelphicus* (*Philadelphia fleabane*), thus excluding it from the site

of action in the chloroplast, either by its binding to unidentified cellular components or by its storage in the vacuole, was strongly advocated by several workers as the major mechanism of resistance (Fuerst and Vaughn, 1990). This theory is based on the limited mobility of paraquat observed in resistant biotypes of certain weed species (Fuerst et al., 1985). It also is based on the rapid recovery of chloroplast functions such as CO_2 fixation and chlorophyll fluorescence quenching in the resistant plants, indicating isolation of the herbicide from its site of action (Fuerst and Vaughn, 1990). Heap et al. (1982) reported that after Hoelon is applied to Italian ryegrass, it floods the tissues in both resistant and susceptible biotypes, inhibiting AcCase activity and depolarizing membranes. It is suggested that the diclofop acid is then sequestered, perhaps in the vacuole or the apoplastic space, such that the concentration of the biocidal components in proximity to the membranes and the AcCase is reduced. Although data demonstrating sequesteration per se are not available, such a postulate would account for the substantial pools of diclofop acid in the tissues, and the observation that the resistant plants continue to grow in the presence of these pools. Rendina et al. (1988) observed that at sufficiently low concentrations of diclofop methyl, the inhibition of AcCase, which is reversible, would be reduced in both biotypes but only the membranes in resistant plants would repolarize. It should be noted that this study was conducted with only one of hundreds of biotypes of resistant ryegrass. Because most cases of resistance have evolved separately, the mechanism of resistance will not necessarily be the same in all populations. It is also probable that populations will contain individuals with different mechanisms of resistance. In a population of

Italian ryegrass (*L. multiflorum*) which is closely related to rigid ryegrass (*L. rigidum*), Stanger and Appleby (1989) observed that AcCase is inhibited less in resistant than in susceptible plants. Gronwald et al. (1989) made the same observation. Rendina et al. (1988) concluded that the only diclofop-related physiological and biochemical differences yet reported between susceptible ryegrass biotype SLR2 and resistant ryegrass biotype SLR31 are: differences in the ability to recover membrane polarization; the 15% higher pool size of diclofop acid in shoots of susceptible plants; and a 15% higher capacity to form conjugates in the shoots of resistant plants. At present, the simplest interpretation of their data is that postulated in Figure 1.

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┌─────────────────────────────────────────────────────────────────┐
│  ┌──────────────┐   ┌──────────────────┐   ┌──────────────┐      │
│  │ Susceptible  │   │ Both susceptible │   │  Resistant   │      │
│  │    SLR2      │   │  and resistant   │   │    SLR31     │      │
│  └──────────────┘   └──────────────────┘   └──────────────┘      │
│                                                                   │
│                       Herbicide floods                            │
│                        into tissue                                │
│                              │                                    │
│                              ↓                                    │
│                                                                   │
│                       AcCase inhibited,                           │
│                     membrane depolarized                          │
│                              │                                    │
│                              ↓                                    │
│                                                                   │
│  Herbicide sequestered              Herbicide sequestered         │
│     some metabolism                    some metabolism            │
│          │                                   │                    │
│          ↓                                   ↓                    │
│                                                                   │
│   Inhibited processes,              Inhibited processes           │
│      do not recover                      recover                  │
└─────────────────────────────────────────────────────────────────┘
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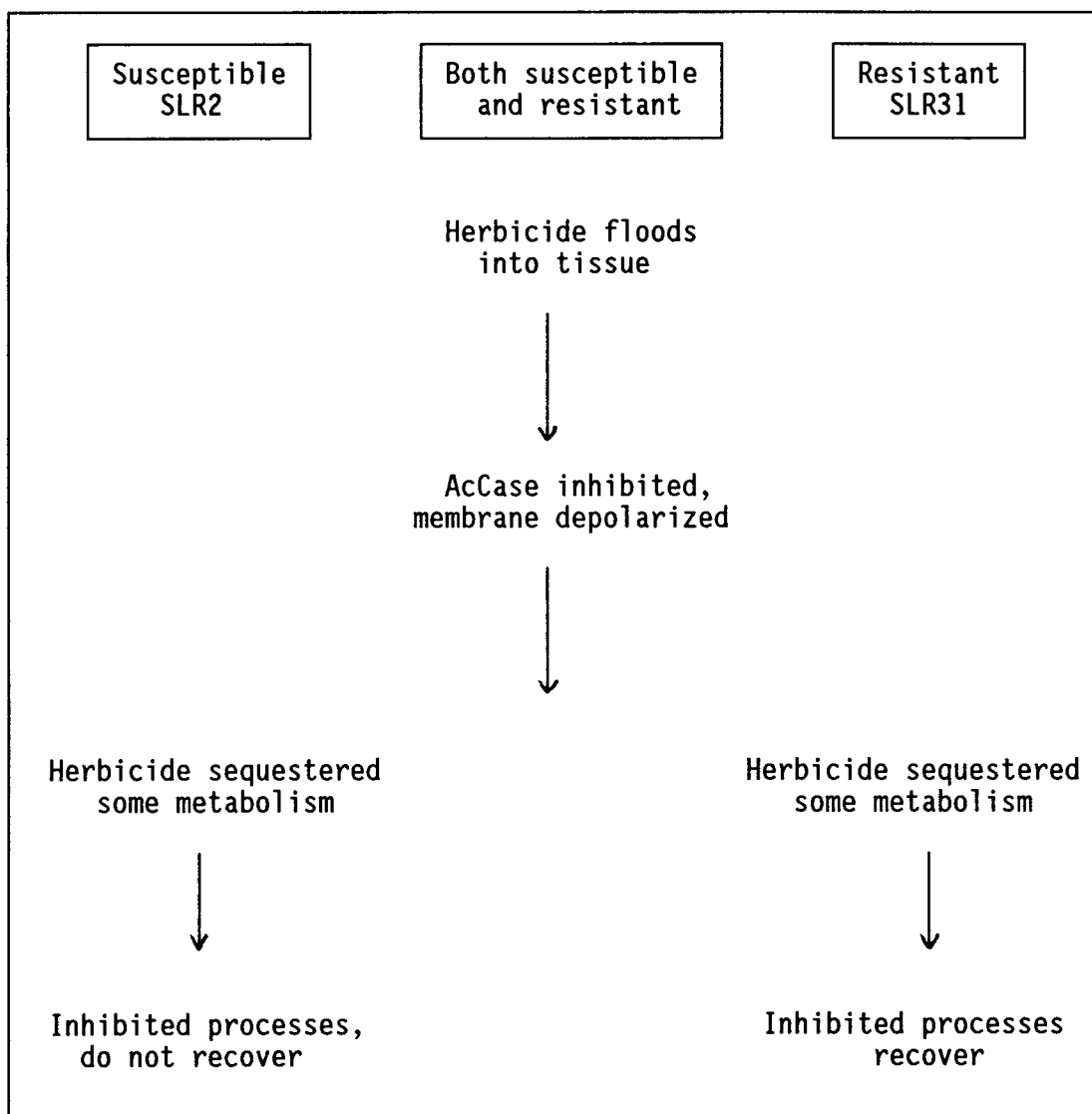
Figure 1. A model that describes the postulated sequence of events that
lead to diclofop-methyl resistance in *L. rigidum* biotype
SRL31. (Adapted from Heap et al., 1982.)

Much progress has been made during the last decade in
understanding the physiological and biochemical basis of herbicide
action. However, further research is needed since the mechanisms of
resistance of a relatively large number of resistant weeds has not yet
been elucidated or studied. From the data accumulated so far, it is

known that a given species can evolve resistance to different herbicides in different geographic locations. Also, resistance to the same herbicide can be based on different mechanisms in different species.

Several attempts have been made to develop models which integrate the genetic, ecological, and physiological processes involved in the evolution of herbicide resistant populations (Maxwell et al., 1990). These models, based on detailed analysis of case histories, highlight the relative importance of factors controlling the development and spread of herbicide resistance. Radosevich et al. (1982) state that these models may indicate how to modify our current practices in order to overcome the already existing resistance problems. They may also serve as predictive tools for evaluation of new management strategies to prevent, or at least delay the appearance of additional problems. These models should provide invaluable information on the effects on weed populations imposed by monoculture and monoherbicide regimes, and the lack of cultivation.

Radosevich et al. (1985) stress the importance of two major processes that determine the dynamics of herbicide resistance: first, processes such as survivorship, fecundity of the pollen and seeds, which influence the relative fitness of resistant biotypes and secondly, the processes that contribute to gene flow in space and time, which alter the frequencies of resistant and susceptible alleles in a population. These factors include immigration of pollen and seeds, seed dormancy, and breeding activities. Although the various models differ in strategy and management approaches, in each case workers concluded that selection pressure must be lessened by reducing rates and frequency of herbicide use.

With reference to fitness and resistant traits, several workers assert that reduced vigor and ecological fitness is an intrinsic feature of the herbicide resistant trait and may be considered as a "cost" or penalty for resistance (Radosevich and Holt, 1982; Gressel, 1985). They reported that the relative fitness of resistant biotypes is highest when a herbicide (or a mixture of herbicides with the same mode of action) with high "effective kill" of the susceptible plants is present. When the selector (e.g. the herbicide) is removed, however, by rotating to another crop or herbicide, the demography of the weed population will inevitably be different. The inferior resistant type will be gradually replaced by the more fit susceptible plants. In the case of triazine-resistant plants, because of alterations in the herbicide binding site, a less efficient electron transport is suggested as the reason for the lower photosynthetic potential, thus reducing vigor and general ecological fitness (Conard and Radosevich, 1979; Radosevich and holt, 1982; and Ahrens and Stoller, 1983).

Other studies, however, suggest that some nuclear control of fitness occurs in triazine-resistant weeds (Stowe and Holt, 1988) which complicates the prediction of fitness. It is quite difficult to predict or quantify the level of selection pressure that leads to resistance. In Australia, resistant rigid ryegrass (*Lolium rigidum*) appeared after only four years using diclofop-methyl, a herbicide with little soil activity, and usually only an annual application. This also occurred with blackgrass (*A. myosuordes*) in the U.K. (Kemp et al., 1990). In both cases, the occurrence of resistance cannot be linked solely with the intensity and amount of herbicide used, suggesting that other factors may be involved in the development of resistance. Both species

are out-crossing, have high reproductive capacity and relatively short-
lived seeds, which may increase their tendency to acquire resistance to
various herbicides.

Several additional models address the initial frequency of the
resistance trait. However, there is ample evidence that because of
polymorphism, resistant genotypes are present in plant populations at
varying frequencies before any exposure to herbicides. Even in more
uniform crops, genetic variations exist as evident by differential
response of crop varieties to herbicides.

2.5 Cross-Resistance

Reports of resistance or shifts towards more insensitive biotypes
in some species, to a few other classes of herbicides predated the
triazine-resistant weeds. However, most of such biotype resistant
biotypes have evolved (Bandeen et al., 1982). At least 50 plant
biotypes are reported to be resistant to 14 classes of herbicides. A
few of these have developed cross-resistance, in which the weed first
evolved resistance after frequent use of one herbicide and was then
found to be resistant to one or more additional types of herbicides that
had not previously been used on the weed (Heap and Knight, 1986;
Gressel, 1988; Solymos and Lehoczki, 1989). LeBaron and McFarland
(1990) noted that the list of 14 other classes of herbicides to which
weed biotypes have evolved resistance includes many of our most
important classes, including some multiple-site-of-action herbicides and
those predicted to be low risk for resistance.

Heap tested 12 populations of Italian ryegrass for cross-
resistance to six herbicides. Each population expressed a degree of
cross-resistance to the other herbicides. The degree of cross-

resistance, however, differed between the populations and was not related to the small differences observed in diclofop-methyl resistance. A variation in cross-resistance suggests that populations which have been selected by exposure to diclofop-methyl and have evolved resistance to other herbicides do not have a single common mechanism for metabolizing the other herbicides and therefore several mechanisms must be involved. By extrapolation, it might also suggest that there may be more than one mechanism for metabolizing diclofop-methyl. A resolution of the issue of how resistance to diclofop-methyl results in cross-resistance to other herbicides will depend on genetic and biochemical studies. In regard to the biochemical nature of resistance, no detectable difference in the uptake and translocation of diclofop-methyl has been shown between resistant and susceptible Italian ryegrass. Recent experiments conducted by Heap et al. (1986) indicate that diclofop-methyl disrupts the membrane integrity of both resistant and susceptible individuals in a similar manner, but resistant plants are able to recover membrane integrity more completely than susceptible plants. This observation, however, does not explain the observed cross-resistance of rigid ryegrass to other herbicides such as chlorsulfuron or trifluralin.

The first reported diclofop-methyl resistant population exhibited cross-resistance to 15 of 22 herbicides tested. These 15 herbicides cover seven herbicide groups and five different modes of action (Harwood et al., 1987). A similar situation of a grass weed, *Alopecurus myosuroides* (blackgrass), having a wide spectrum of herbicide cross-resistance was reported by Moss and Cussans (1987). The general conclusion was that if cross-resistance had been consistent across

populations, farmers could have been informed which herbicides would control blackgrass. In view of the variation between populations, advice to farmers could only be given after testing their populations for cross-resistance.

With reference to development of cross-resistance with similar mode-of-action herbicides it cannot be assumed that different herbicide classes with the same mode of action will be equally ineffective in killing a resistant weed population. Weeds can be resistant to a herbicide in one subclass of a particular chemistry but not resistant to another subclass within that same chemistry. Sulfonylurea-resistant weeds may be, but are not necessarily, cross-resistant to the imidazolinones. In studies conducted by American Cyanamid, five sulfonylurea-resistant kochia biotypes were tested. Of those, only one kochia biotype was found to be cross-resistant to some of the imidazolinones. In addition, sulfonylurea-resistant Russian thistle and common chickweed were not cross-resistant to imidazolinones (Primiana et al., 1990). It becomes apparent that response by weeds may vary for each individual compound. Although the imidazolinones and sulfonylureas both inhibit the activity of an essential plant enzyme, acetolactate synthase or acetohydroxyacid synthase (ALS or AHAS), these herbicides bind to different sites on the enzyme. These differences in binding sites explain why species resistant to the sulfonylureas may not be cross-resistant to the imidazolinones. Theoretically, there is a greater potential for a weed population to develop resistance to two similar mode-of-action herbicides than to two dissimilar mode-of-action herbicides. However, it cannot, and should not, be assumed that cross-resistance will occur (Primiana et al., 1990).

2.6 Management of Herbicide-Resistance

The fact that herbicide resistance is predominantly associated with monocultural crops, intensive use of herbicides, and reduced cultivation or no-tillage practices, any attempt to delay or prevent resistance must include measures that address these practices. Rational use of herbicides has been one of the proposed approaches by several researchers. These include the alternative use of herbicide rates which involves increased herbicide dose and/or a switch to alternative herbicides while maintaining other normal practices. This approach was found to be the simplest response of a farmer, upon detection of a reduced performance or failure of any herbicide. Such an approach might solve the problem temporarily as suggested by Stephenson et al. (1990), but is bound to fail in the long run. This was the case with insecticide-resistance which led to the "pest treadmill" phenomenon (Ruscoe, 1987).

Stephenson et al. (1990) also suggested using mixtures of herbicides having different modes of action as a primary preventative measure, both to avoid or delay and to reverse herbicide resistance. However, Radosevich et al. (1982) contended that using such mixtures will obviously lower the initial frequency of the R type and broaden the weed spectrum controlled, but it may also result in elevated selection pressure. However, the feasibility of this approach is further reduced by cross- and multiple-resistance, a major problem which complicates the battle against herbicide resistance.

Use of herbicide synergists and safeners has been proposed by many researchers. Varsano (1987) reported that the application of a known cytochrome P_{450} inhibitor, such as piperonyl butoxide (PB) and 1-

aminobenzotriazole (ABT) in combination with several herbicides improved their efficacy in controlling resistant weeds. Varsano and Rubin (1980) further suggested that the observed synergy of PB with atrazine in triazine-resistant ryegrass might be attributed to a rapid, and light-dependent, damage to membrane integrity caused by the combination. Though the practical implementation of synergists in weed management programs is very limited at present, it may allow a reduction in herbicide rate which is economically and environmentally desirable.

Based on their simulation models, Radosevich and coworkers (Maxwell et al., 1990; Roush et al., 1990) have suggested a different approach in the management of herbicide resistance. The authors espouse the importance of optimizing herbicide input to the economical threshold level by restricting herbicide rates to low levels and deliberately leaving some weeds in the field, and along its margins. This would allow the susceptible plants to compete with the resistant plants, further diluting their proportion of the weed population through fitness and gene flow via seeds and pollen.

MATERIALS AND METHODS

3.1. Field Experiment

A field experiment was conducted at the Hyslop Agronomy Farm of Oregon State University, October 8, 1991 to April 10, 1992. The area was prepared mechanically by one plowing and three disc harrowings. Experimental plots measured 1x2 meters. Seeds of accessions of Italian ryegrass collected from 10 locations within the Willamette Valley in Western Oregon were provided by the Department of Crop and Soil Science at Oregon State University. These seeds were planted on October 11, 1991, at the rate of 4 grams (approximately 500 seeds/m^2) per plot spaced along a 50-75 cm strip within the plot. Sprinkler irrigation (2.0 cm water) followed planting to ensure adequate moisture for germination.

Eight herbicides (Table 1) were applied preemergence soil incorporated or preemergence or postemergence, representing main plots. Subplots consisted of 10 different accessions of Italian ryegrass arranged randomly within main plots, to form a split plot design.

Table 1. Characteristics of the tested herbicides.[a]

Herbicide	Adsorption	Soil Degradation	Vapor Pressure (mg Hg)	Mechanism of Action	Absorption	Application
Diuron	Clay/O.M.	Microbes	0.31×10^{-5}	Inhibitor of the hill reaction	Root	PRE, DPE
Pronamide	O.M.	Microbes	8.5×10^{-5}	Mitotic inhibitor	Root	PRE, POST
Diclofop	--	Decomposes under aerobic conditions	$.258 \times 10^{-6}$	AcCase inhibitor	Leaves	POST
Metribuzin	Clay and/or O.M.	Microbes	$< 1 \times 10^{-5}$ at 20°C	Photosynthetic inhibitor	Roots	PRE, EPE
Triallate	Soil colloids	Microbes	1.2×10^{-4} at 25°C	Inhibits cell division	Coleoptile	PP1, PE1
Trifluralin	Clay	Microbes	1.1×10^{-4} at 25°C	Affects physiological cell growth processes associated with seed germination	Shoot	PP1, PE1
UB1 C4243	N/A	N/A	N/A	N/A	N/A	N/A

[a] Source: Herbicide Handbook of the Weed Sci. Soc. of Amer. 1983. Fifth Edition.

3.1.1. Treatment Specifications

i) Preemergence surface applied herbicides

Common name	Trade name	Form	Conc G/L or % a.i.	Rate kg/ha a.i.
a) Pronamide	Kerb	WP	50.00	1.1
b) UB/C4243	--	L	100.00	0.07

ii) Preemergence incorporated herbicides

Common name	Trade name	Form	Conc G/L or % a.i.	Rate kg/ha a.i.
a) Triallate	Fargo	L	480.00	1.4
b) Trifluralin	Treflan	L	480.00	0.8

iii) Postemergence herbicides

Common name	Trade name	Form	Conc G/L or % a.i.	Rate kg/ha a.i.
a) Pronamide	Kerb	WP	50.00	1.1
b) Metribuzin	Lexone	df	75.00	0.6
c) Diclofop-methyl	Hoelon	L	360.00	1.1
d) Diuron	Clean Crop	df	80.00	1.8

iv) Check (no herbicide)

The preemergence and the preemergence incorporated herbicide treatments were executed one day after planting, using a unicycle sprayer equipped with flat fan nozzles delivering 475 L/ha. The postemergence herbicides were applied 25 days later using the same type of sprayer. Plants at this time had attained the desirable 2-3 leaf stage. One block per replication was herbicide-free to serve as a control.

Fertilizer (16-12-0) at the rate of 300 lb/acre was applied and incorporated one day before planting. Temperature and precipitation data are given in Table 2.

Table 2. Mean monthly temperature (T) and precipitation during the
field experiment period at Hyslop Agronomy Farm (1991-1992).[a]

Month	Year	Rainfall (inches)	T° (F) Max	Min
October	1991	2.55	68.8	40.8
November	1991	5.13	53.4	41.2
December	1991	4.38	46.3	36.1
January	1992	4.52	49.5	37.2
February	1992	4.54	56.1	38.7
March	1992	4.55	55.0	37.0

[a] Source: Oregon Climate Service, Oregon State University, Corvallis,
Oregon. 1992.

3.1.2. Environmental Conditions at the Time of Treatment

	Preemergence Application (October 12, 1991)	Postemergence Application (November 2, 1991)
Temperature (F)	Air: 44; Soil: 55	Air: 41; Soil: 52
% Relative humidity	78	76
% Cloud cover	0	0
Wind speed and direction	Calm	Calm
Dew present	Yes	Yes
Time of day	7:30 a.m.	9:00 a.m.
Soil moisture	Dry	Wet
Soil surface	Fine	Fine

3.1.3. Data Collected

Control was evaluated every three weeks beginning three weeks after herbicide application. Percent control was based on visually integrating stand reduction and growth inhibition for each treatment in comparison to the respective untreated check. Plants were harvested 12 weeks after planting and shoot fresh weights were recorded. Means of

fresh weights were converted to percent of untreated check. Mean percent control at 3, 6, 9 and 12 weeks after planting were calculated. Differences among accessions were determined by analysis of variance (ANOVA) and multiple range tests, when appropriate. All statistical analysis were conducted using PC-SAS software.

4.1. Greenhouse Experiment

4.1.1. Sources of Italian Ryegrass Seeds

Italian ryegrass seeds were collected from 10 locations within the Willamette Valley in Western Oregon. Seed from a given location will be referred to as "an accession" henceforth in this paper.

An accession of commercial ryegrass seed was collected from a field with no history of herbicide treatments. This was used as a standard susceptible stock since its sensitivity to Hoelon (diclofop-methyl) was similar to that of other non-resistant populations. The remaining nine accessions were collected from fields where pronounced resistance to Hoelon was identified in the past years.

4.1.2. Conduct of the Experiment

The experiment was conducted in the greenhouse of the Crop and Soil Science Department, Oregon State University, in the spring of 1992. Ten accessions of Italian ryegrass (*Lolium multiflorum*) were studied, using respective field rates of selected herbicides from various herbicide families with similar and/or dissimilar modes of action to/from Hoelon. Twenty seeds were sown at a depth of 1 cm in 10 square centimeter plastic pots containing Woodburn silt loam soil to a depth of 8 cm.

4.1.3. Preemergence Application of UBI C4243 and Pronamide

The effect of UBI C4243 and pronamide on one commercial (susceptible) and nine supposedly diclofop-resistant Italian ryegrass accessions from 10 locations was studied in this experiment. Pronamide and UBI C4243 1.1 kg ai/ha and 0.07 kg ai/ha, respectively, were applied preemergence. This application was done on March 6, 1992.

Sowing dates were arranged so that all treatments were applied on the same day. Commercial formulation of the herbicides in question were applied using the greenhouse sprayer equipped with an 80015E flat fan spray tip delivering 269 L/ha at 26 psi CO_2. Following herbicide application, pots were placed in the greenhouse with an initial overhead irrigation, then subsequently sub-irrigated as necessary. The greenhouse had supplementary heating and lighting to provide a 20C 16-hour day and 13C, 8-hour night. Percent control was estimated visually 3 and 6 weeks after treatment (WAT) compared with the check, and foliage fresh weights at harvest were recorded.

4.1.4. Foliar Application of Diclofop-methyl, Diuron, Metribuzin and Pronamide

The one susceptible and the nine accessions supposedly resistant to diclofop were evaluated for cross or multiple resistance to diuron, metribuzine and pronamide. Seeds of the ten accessions were germinated in plastic pots containing 8 cm of potting soil on March 6, 1992. The pots were placed on wooden benches in the greenhouse. At the second to third leaf stage, 21 days after sowing, a single field rate of each herbicide was applied.

The treatments were applied using a greenhouse sprayer equipped with an 80015E flat fan spray tip delivering 269 L/ha at 26 psi CO_2.

The greenhouse temperature was 20/13C day/night. The pots were provided with water regularly through sub-irrigation. Percent control compared with the check was visually estimated 3 and 6 weeks after treatment and foliage fresh weight was recorded at harvest. Techniques similar to those described by Heap and Knight (1982; 1986) were used to evaluate cross-resistance. Differences among accessions were determined by analysis of variance (ANOVA) and multiple range tests, when appropriate.

RESULTS AND DISCUSSION

5.1. Field Experiment

Figure 2 represents the fresh weight of all accessions when no herbicide was applied to them. Fresh weights at harvest ranged from 180 to 280 grams per .18 m^2 (i.e. 9,682 - 15,000 kg/ha) for the different accessions. Accession 6 has the highest fresh weight followed by the commercial accession (accession 10). There is no significant difference in fresh weight between accessions 2, 3 and 7. Fresh weights for accessions 1, 4, 8 and 9 are not significantly different from each other. Accession 5 had the least fresh weight.

CHECK - NO HERBICIDE
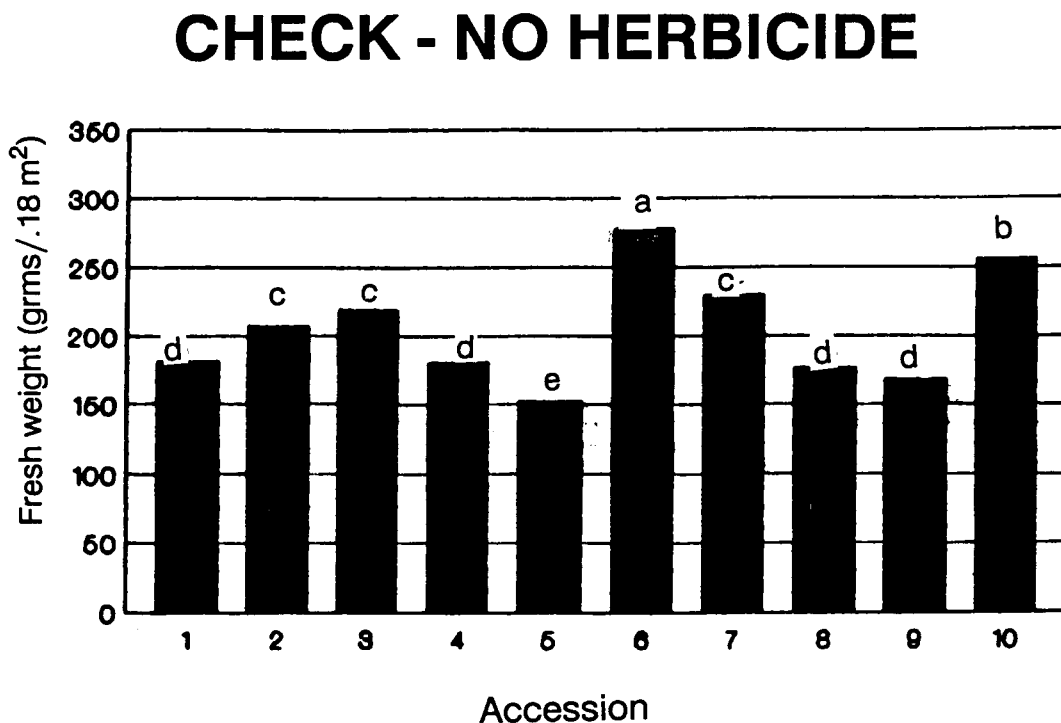
LSD .05 = 15.66

Figure 2. Fresh biomass weight of accessions of Italian ryegrass without herbicide treatment.

5.1.1. <u>Preemergence Surface Applied and Preemergence Soil
Incorporated Herbicides</u>.

Figure 3 represents the response of accessions of Italian ryegrass

(*Lolium multiflorum*) to preemergence application of pronamide. There

were no significant differences in percent control for any of the

accessions compared with the check at a field rate of 1.1 kg/ha of

pronamide. One hundred percent control was achieved as early as four

weeks after application, and maintained throughout the rest of the

experimental period.

PRONAMIDE
PREEMERGENCE
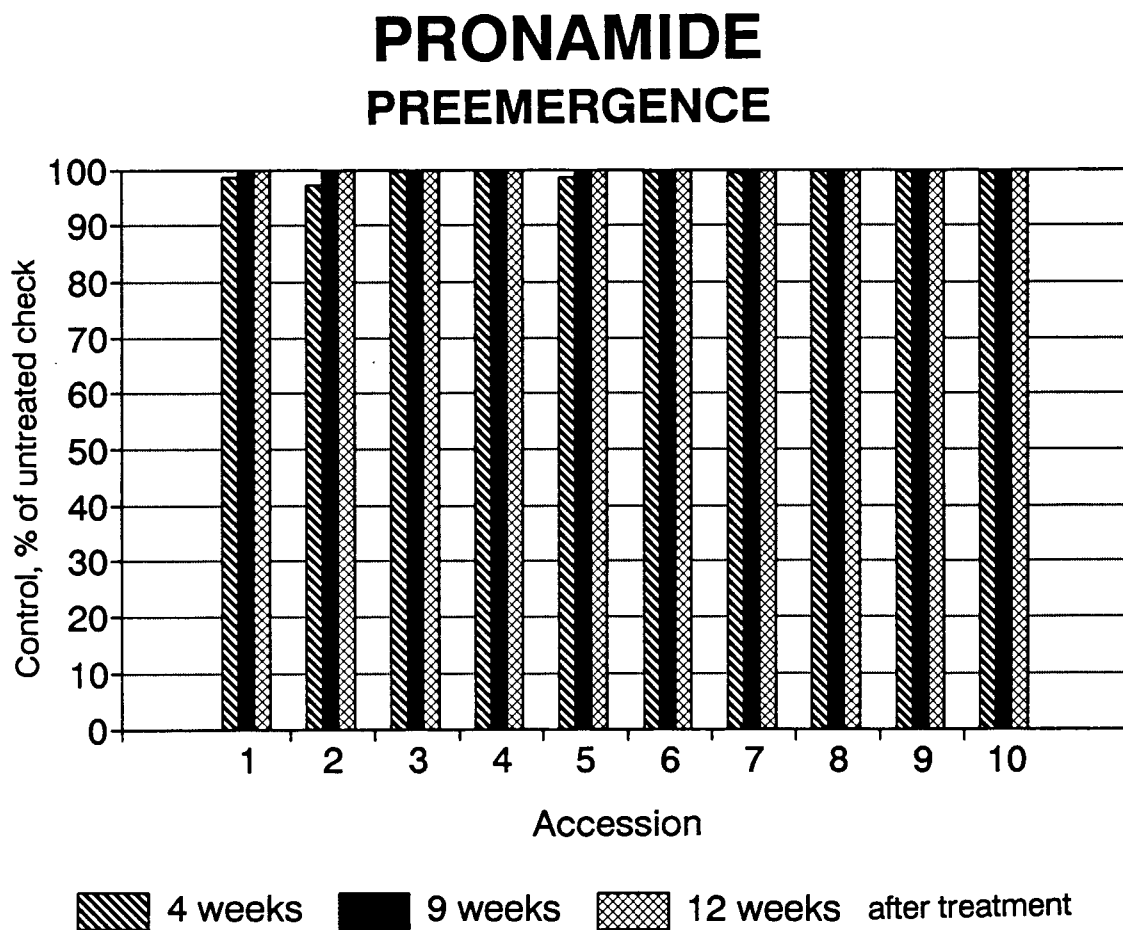
Figure 3.   Percent control of accessions of Italian ryegrass (*Lolium
multiflorum*) with 1.1 kg/ha of pronamide preemergence.

Four weeks after application of 0.07 kg ai/ha of UBI C4243,

percent control of all accessions was approximately 80% (Figure 4).

However, percent control increased to over 90% for all accessions by the
end of the ninth week. This is a high degree of efficiency of this
product at such a low rate. Fresh weights at harvest were the highest
for accessions 7 and 10, followed by accession 4 (Figure 5). Fresh
weights were reduced from 96.8 to 99.3% in response to UBI C4243
application compared with corresponding checks.

UBI C4243
PREEMERGENCE
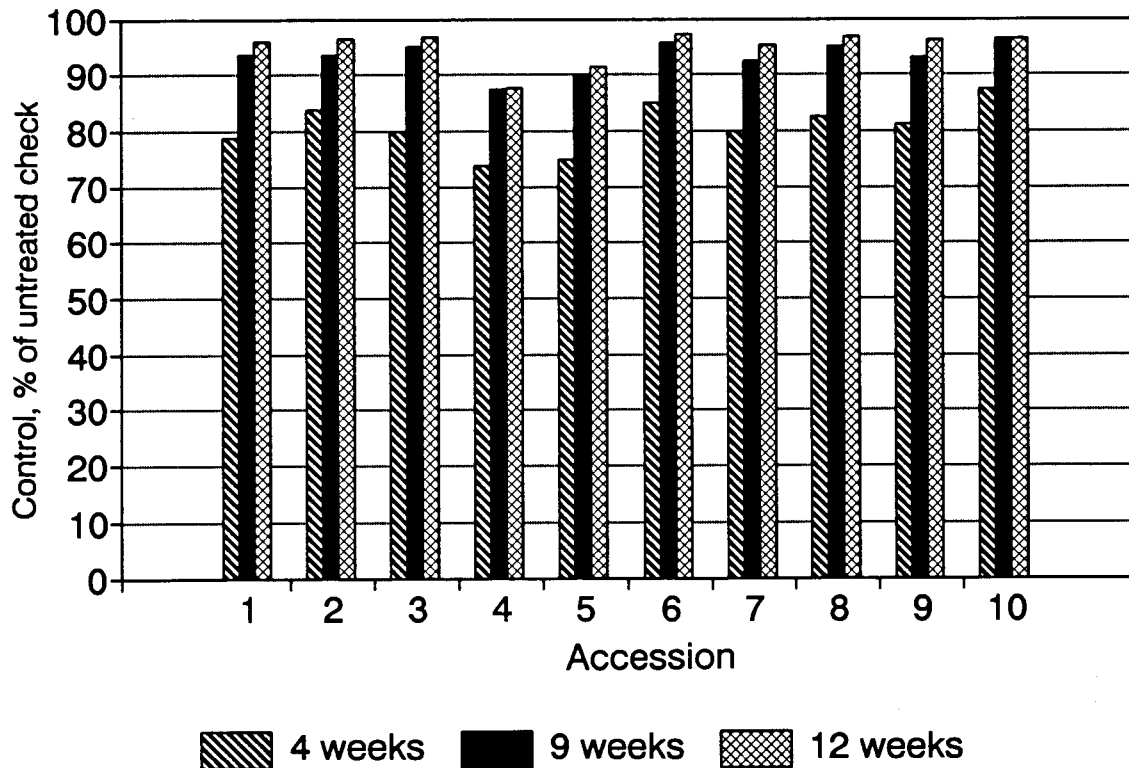
Figure 4.   Percent control of accessions of Italian ryegrass (*L.
            multiflorum*) with 0.07 kg ai/ha of UBI C4243 preemergence.

UBI C4243
PREEMERGENCE
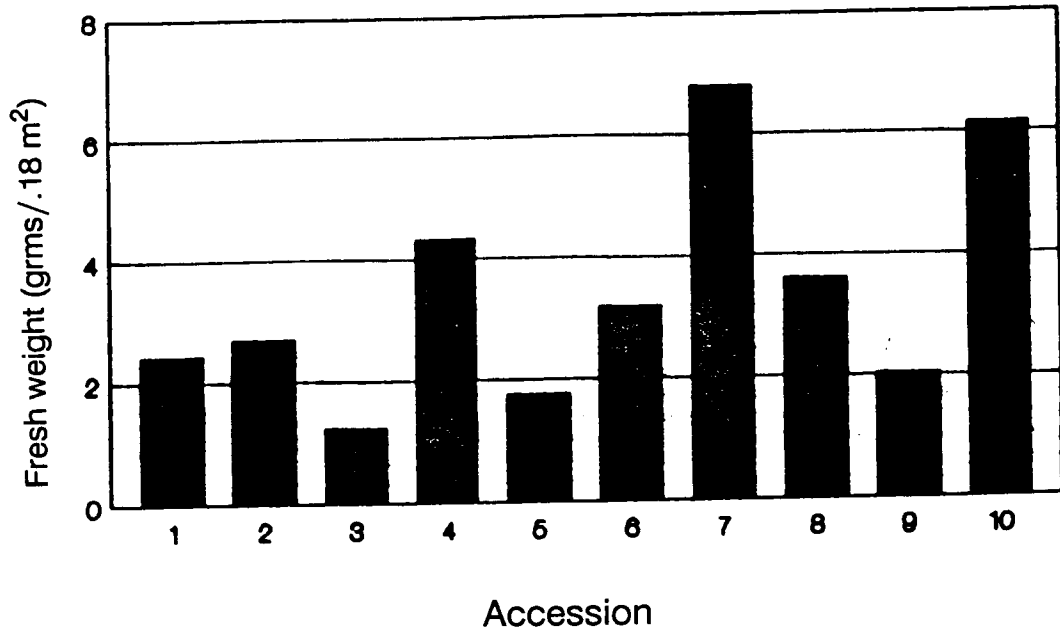
Figure 5. Fresh biomass weight of accessions of Italian ryegrass (*L. multiflorum*) in response to UBI C4243 applied preemergence.

There were no significant differences in percent control between accessions or when compared with their respective checks for preemergence-incorporated application of both triallate and trifluralin. Respective field rates were 1.4 kg/ha and 0.8 kg/ha (Figures 6 and 7). Mean control was 98% 12 weeks after treatment for all the accessions with application of triallate except for accessions 8 and 9, both with a control of 80%. Control with trifluralin was 98% for all accessions for the corresponding period.

TRIALLATE
PREEMERGENCE
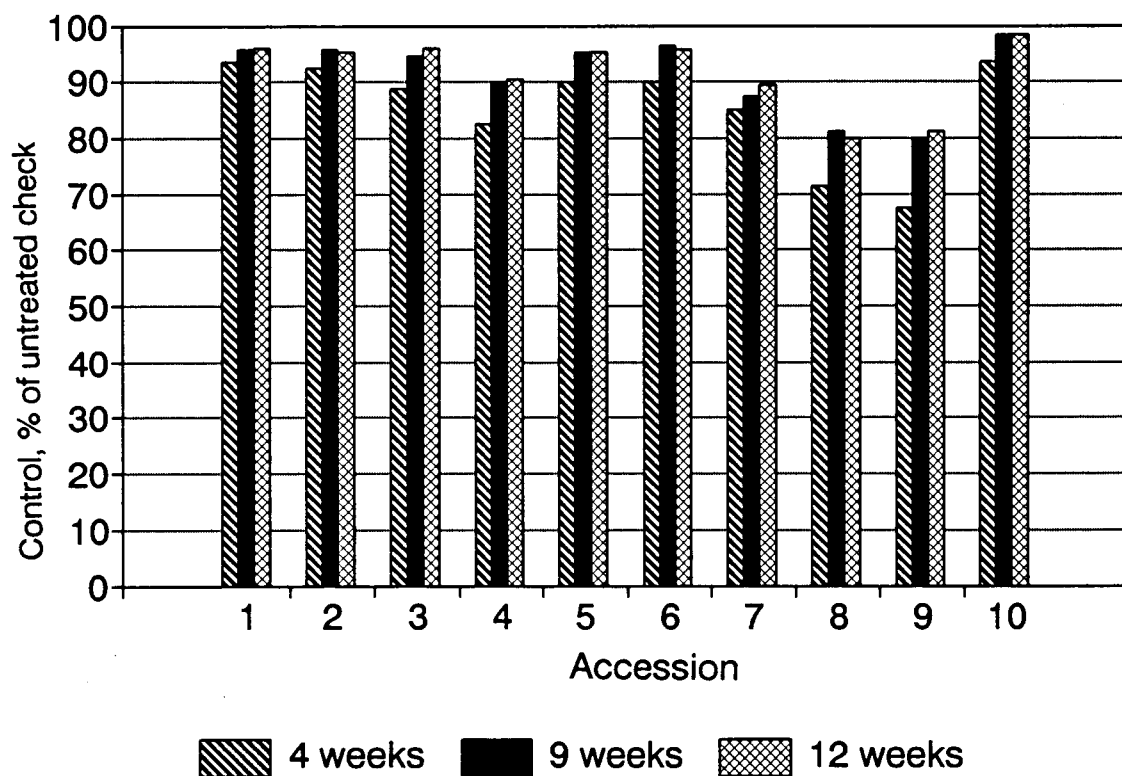
Figure 6. Percent control of accessions of Italian ryegrass with
1.4 kg/ha of triallate preemergence incorporated.

TRIFLURALIN
PREEMERGENCE
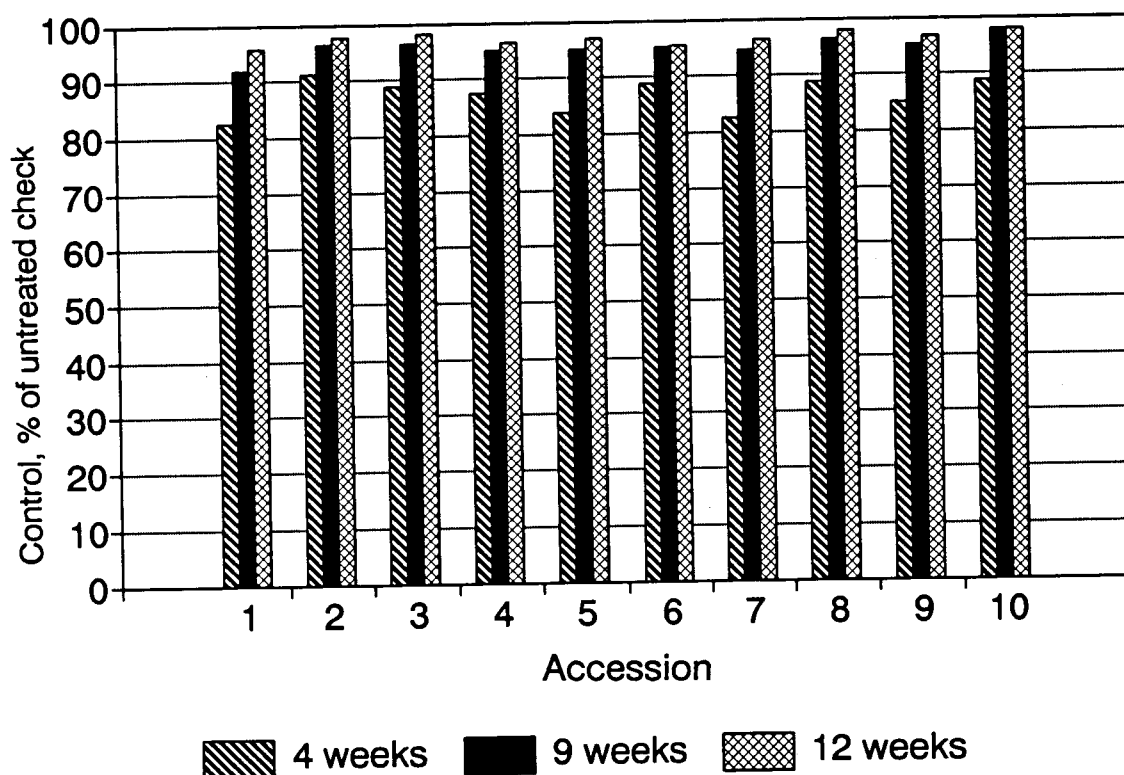
Figure 7. Percent control of accessions of Italian ryegrass with
0.8 kg/ha of trifluralin preemergence incorporated.

Fresh weight at harvest for the various accessions of Italian ryegrass with preemergence-incorporated applications of triallate and trifluralin did not differ significantly. Compared with the zero herbicide treatment, reduction in biomass ranged from 96.2 to 98.4% for both herbicides at harvest (Figures 2, 8 and 9).

TRIALLATE
PREEMERGENCE INCORPORATED
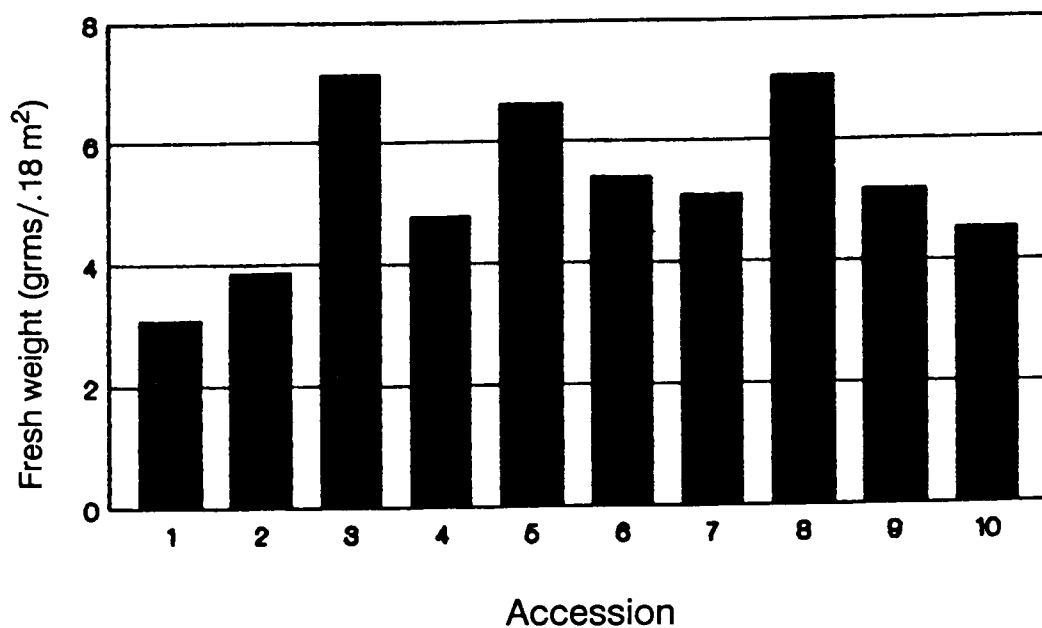
Figure 8. Fresh biomass weight of accessions of Italian ryegrass in response to 1.4 kg/ha of triallate preemergence-incorporated.

TRIFLURALIN
PREEMERGENCE INCORPORATED
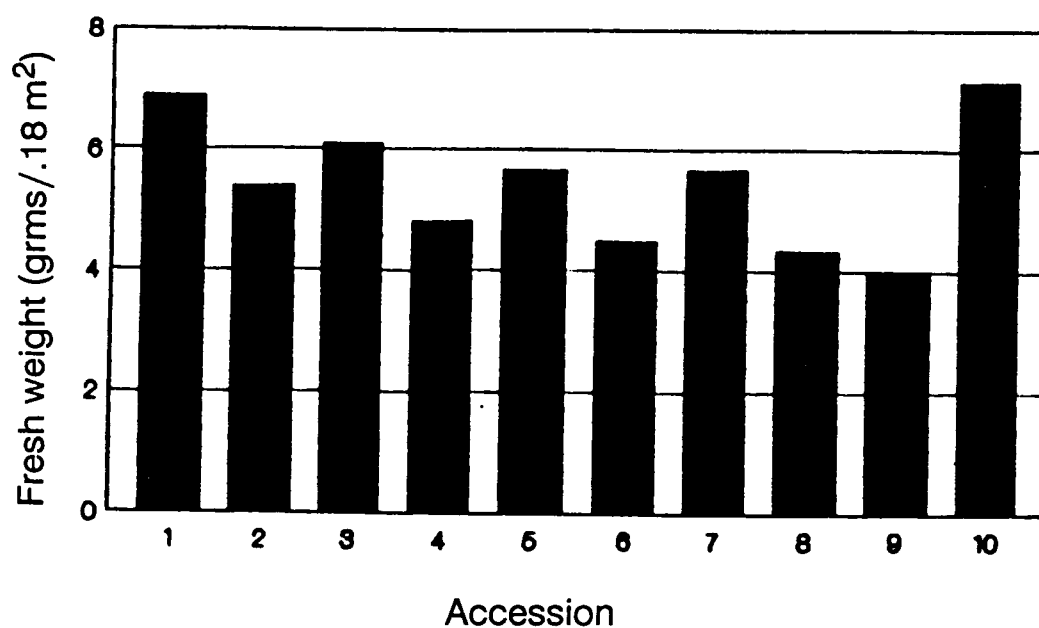
Figure 9. Fresh biomass weight of Italian ryegrass in response to 0.8 kg/ha of triafluralin preemergence-incorporated.

5.1.2. Postemergence Herbicides

Percent control three weeks after a postemergence application of diuron, varied dramatically for accessions 4 and 8 (Figure 10). Percent control ranged between 85 and 95% for all accessions excluding the resistant accessions 4 and 8 for which control was approximately 3 and 10%, respectively. This expression of tolerance or resistance to 1.8 kg/ha of diuron postemergence, persisted for the 12 week duration of the experiment. Control of diuron-resistant accessions 4 and 8 was comparable to control with Hoelon of accessions 1 through 6 and 8 (Figure 10). However, accessions 7 and 9 were moderately resistant to Hoelon with percent control of 30 and 58%, respectively. Accession 10 (commercial population) was highly susceptible to Hoelon; 100% control at 1.1 kg/ha.

DIURON
POSTEMERGENCE
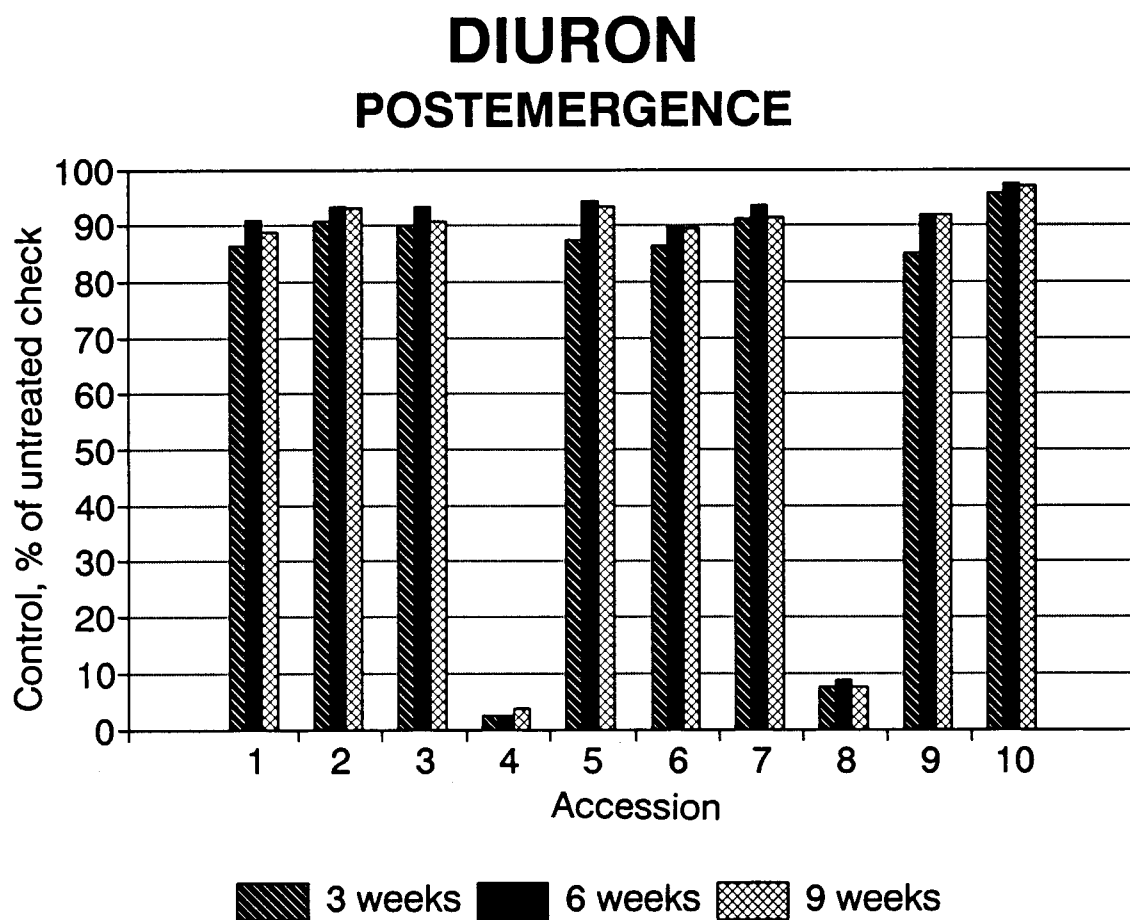
Figure 10. Control of different accessions of Italian ryegrass with 1.8 kg/ha of diuron applied postemergence.

DIURON
POSTEMERGENCE
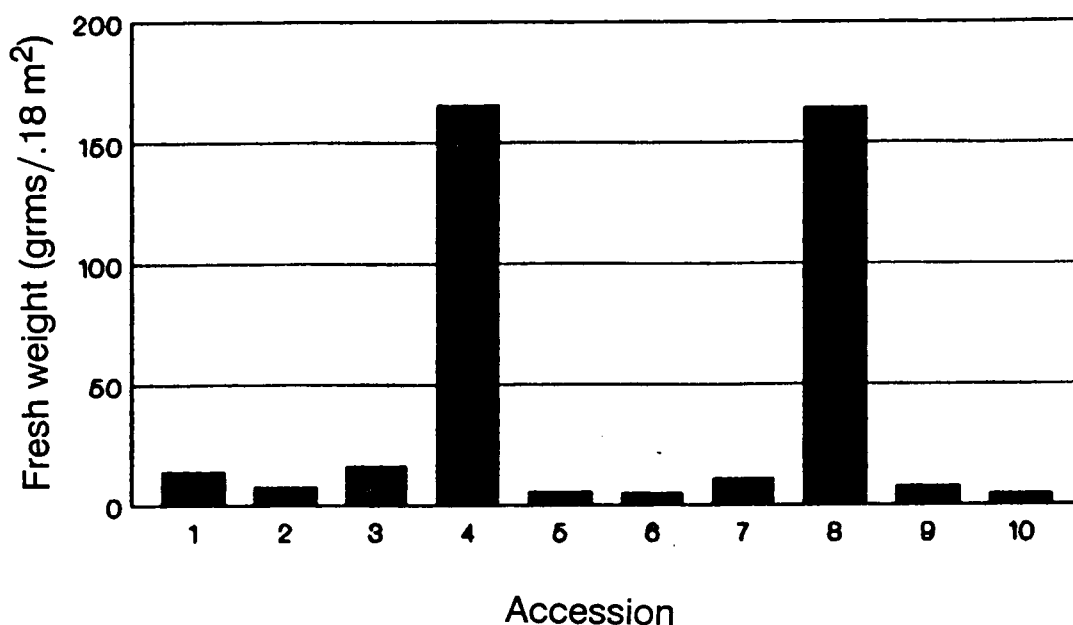
Figure 11. Fresh biomass weight of accessions of Italian ryegrass in
response to 1.8 kg/ha of diuron applied postemergence.

Among postemergence treatments, metribuzin at 0.6 kg/ha controlled
the 10 accessions of Italian ryegrass most effectively. One hundred
percent control of all accessions was achieved within six weeks of
metribuzin application (Figure 12). In metribuzin-treated plots, almost
all gramminaeceous weed seedlings, such as volunteer annual bluegrass,
were killed. However, a few broadleaf weeds such as Canada thistle
(*Cirsium arvense* (L.)) were not controlled by metribuzin.

In response to the application of diclofop-methyl, control of all
the supposedly diclofop-resistant accessions, except for accessions 7
and 9, ranged from 0-7%, confirming resistance to this product at an

application rate of 1.1 kg/ha (Figure 10). Control of accessions 7 and
9 was only 30 and 58%, respectively, suggesting they are moderately
resistant to diclofop-methyl. These results agree with those obtained
by Heap et al. (1982), that development of cross or multiple resistance
in *Lolium multiflorum* is predominantly biotype dependent. Thus, in
resistance management programs, processes or production practices that
seemingly lead to the prevalence of these biotypes, should be re-
examined and modified to retard or avoid development of resistance.
Control of the commercial accession with diclofop was 100% within three
weeks after treatment.

DICLOFOP-METHYL
POSTEMERGENCE
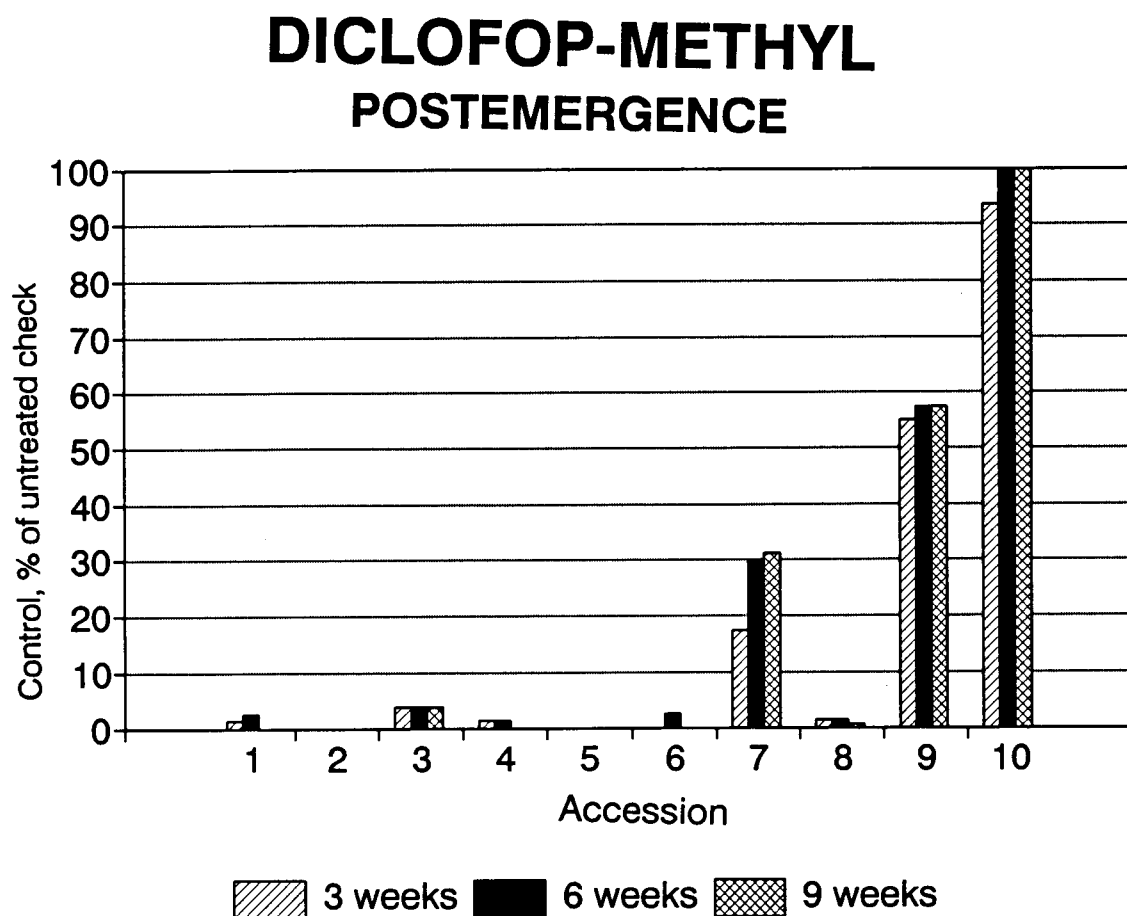
Figure 12. Percent control of accessions of Italian ryegrass with
1.1 kg/ha diclofop-methyl applied postemergence.

Percent control of all accessions with postemergence application of pronamide was consistently low four weeks after application. Nine weeks after treatment, percent control ranged between 62 (accession 2) and 78% (accession 3) (Figure 11). Percent control increased significantly over time with 1.1 kg/ha pronamide postemergence. Control may have increased with more time since the mechanism of action of this product is primarily a cell division inhibitor, a process that can be slow. Nevertheless, even six weeks after treatment, surviving plants appeared necrotic and irreversibly weak.

PRONAMIDE
POSTEMERGENCE
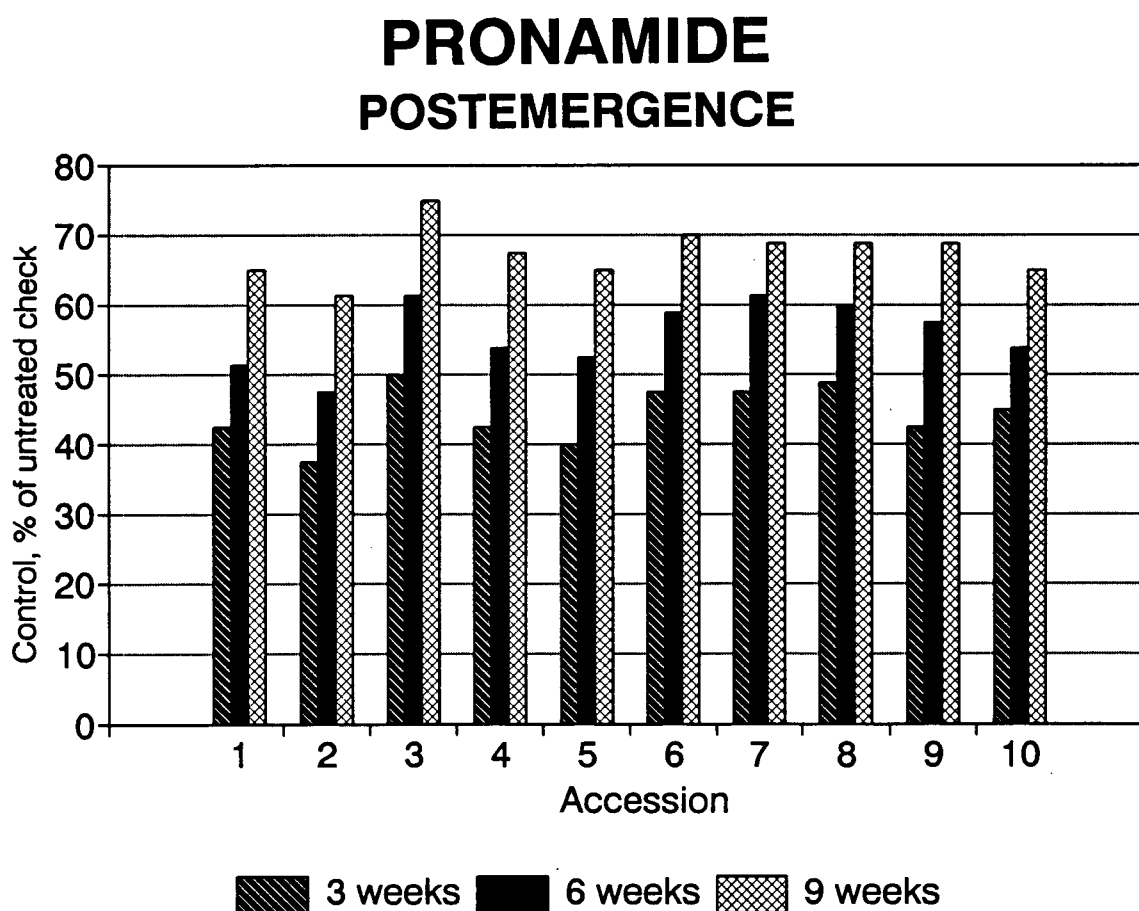
Figure 13. Percent control of accessions of Italian ryegrass with 1.1 kg/ha of pronamide applied postemergence.

METRIBUZIN
POSTEMERGENCE
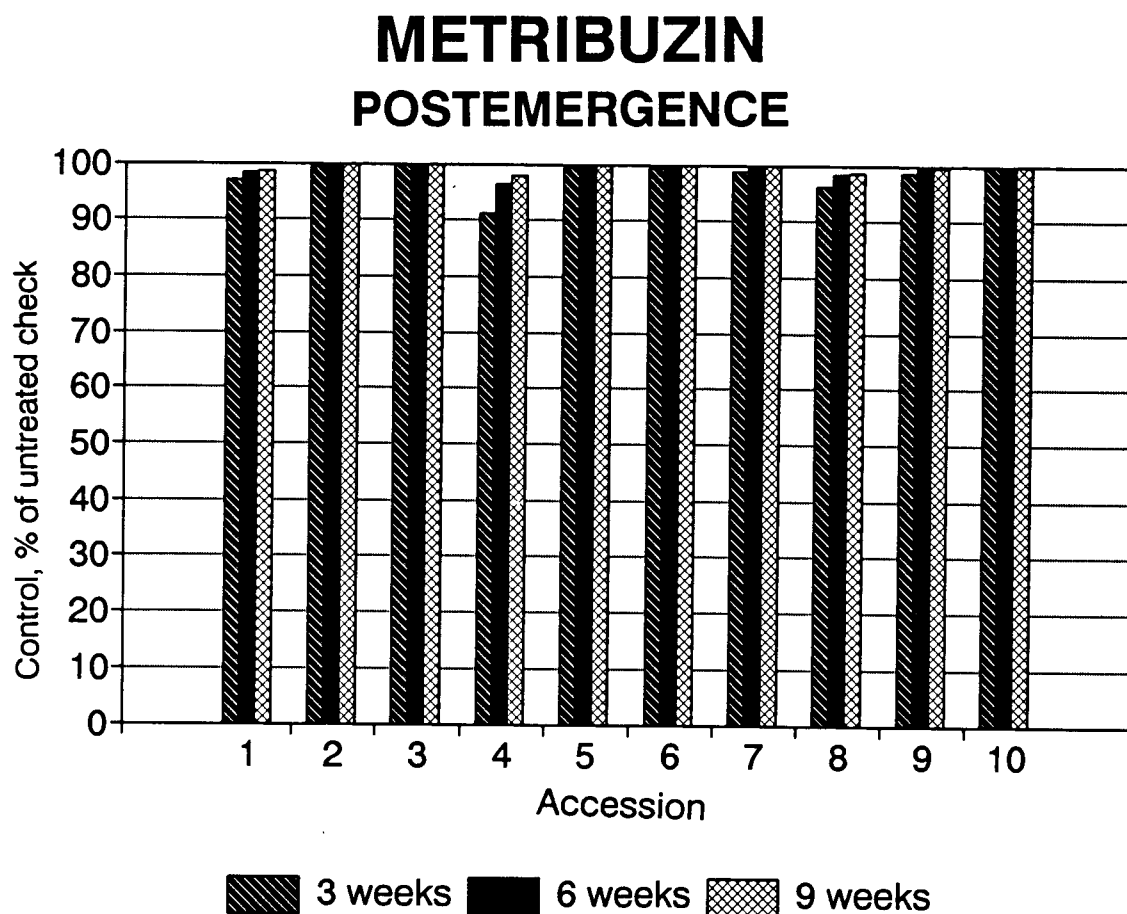
Figure 14. Percent control of accessions of Italian ryegrass with 0.6 kg/ha of metribuzin applied postemergence.

6.1 Greenhouse Experiment

6.1.1. Percent Emergence

Percent emergence differed widely among accessions. Emergence of the diuron resistant accessions and accession 6 was significantly lower than all other accessions (Table 3). However, additional research is needed before conclusions could be made about the fitness of these accessions.

Table 3. Percent emergence of 10 accessions of Italian ryegrass (*L. multiflorum* L.) in the greenhouse.

Accessions	Mean percent germination
1	70.0^e
2	78.3^c
3	70.0^e
4	65.0^f
5	73.3^d
6	66.7^f
7	91.7^a
8	50.0^g
9	83.3^b
10 (commercial)	93.3^a

LSD .05 = 2.56

6.1.2. Preemergence Surface Applied and Preemergence Soil Incorporated Herbicides.

In a greenhouse study with ten accessions of Italian ryegrass (Lolium multiflorum), significant differences were not detected in percent control for any of the accessions compared with their respective check, following a preemergence application of 1.1 kg/ha of promanide. Six weeks after treatment, control was 100% for all but accessions 7 and 9 with 98.7 and 99.3% control, respectively. This response was similar to that obtained in the field where 100 percent control was achieved four weeks after treatment (Table 4).

Three weeks after treatment with 0.07 kg ai/ha of UBI C4243 control of all accessions exceeded 95 percent under greenhouse conditions (Table 4) As commonly experienced with many herbicides (A.P. Appleby, personal communication), control in the greenhouse was greater than that observed for respective treatments under field conditions.

There was no significant difference in percent control between accessions or when compared with the commercial accession for

preemergence incorporated applications of both triallate and trifluralin.

Mean percent control was 94 percent at 6 weeks after treatment for all accessions with triallate PPI except accessions 8 and 9, both with less than 90 percent control. This pattern is similar to the field results obtained on corresponding treatments, except that control increased some 17 percent in the greenhouse. Control with trifluralin was 100 percent for all accessions except for 7 and 9, both with 96% control.

Table 4. Mean percent control at 3 and 6 weeks after treatment and fresh weight of accessions of
Italian ryegrass (*L. multiflorum* L.) treated with preemergence and preplant soil
incorporated herbicides (Greenhouse Experiment).

| Accession | Pron | Mean % Control (3 WAT) | | | Pron | Mean % Control (6 WAT) | | | Pron | Mean FW/pot | | |
		UBI	Tria	Trif		UBI	Tria	Trif		UBI	Tria	Trif
1	100	98	93.3	100	100	99	99.3	100	0	0	0	0
2	100	100	93.3	98.3	100	100	99.3	100	0	0	1.6	0
3	100	100	96.7	96.7	100	100	95	100	0	0	1.7	0
4	100	95	95	100	100	92.7	100	100	0	0	0	0
5	100	98	96.6	96.6	100	98	99.3	100	0	0	.1	0
6	100	98	96.7	100	100	100	100	100	0	0	0	0
7	95	93	75	93.3	99	99	80	96	3.9	1.95	3.7	5.1
8	100	98	85	98.3	99	99	86.7	100	0	0	4.4	0
9	98	98	55	98.3	100	100	86.7	96	0	0	2.2	5.8
10	100	100	96.7	100	100	100	100	100	0	0	0	0

<u>Legend</u>:

Pron = Pronamide
UBI = UBI C4243
Tria = Triallate
Trif = Trifluralin

6.1.3. Postemergence Herbicides.

Percent control three weeks after a postemergence application of diuron ranged between 85-97% for all accessions except 4 and 8, for which control was 6.6 and 5 percent, respectively (Figure 15). Percent control decreased by an average of 50% from the 3rd to the 6th week after treatment. This expression of tolerance to 1.8 kg/ha of diuron by accessions 4 and 8 also existed in the greenhouse.

Figure 15. Mean percent control 3 and 6 WAT and fresh weight 6 WAT of 10 accessions of Italian ryegrass (*L. multiflorum*) with diuron.

Metribuzin at 0.6 kg/ha did not effectively control any accession in the greenhouse. However, metribuzin applied postemergence at this rate controlled ryegrass effectively under field conditions (Figure 16). These results are contrary to those usually expected. Perhaps the

differential water management between the two environments may be responsible for these results. In the greenhouse, subirrigation of the young plants grown in plastic pots was used after the foliar application of the herbicides. Field moisture was dependent on rain which may have washed the herbicides from the leaves into the soil. The primary site of uptake of metribuzin is the roots. Possibly reducing root uptake in the greenhouse by subirrigation could certainly affect the herbicidal activity of metribuzin.

Figure 16. Mean percent control at 3 and 6 WAT and fresh weight 6 WAT of 10 accessions of Italian ryegrass (*L. multiflorum*) after treatment with metribuzin.

All supposedly diclofop-resistant accessions, except for accessions 6, 7 and 9, exhibited resistance to 1.1 kg/ha of diclofop in both the greenhouse and the field. Control of accessions 6, 7 and 9 was 31, 51 and 13 percent, respectively (Figure 17).

Figure 17. Mean percent control at 3 and 6 WAT and fresh weight 6 WAT of 10 accessions of Italian ryegrass (*L. multiflorum*) treated with diclofop-methyl.

Percent control with pronamide was consistently low for all accessions. Control of the commercial population was controlled 41 percent by the product 6 weeks after treatment. Percent control did not exceed 30 percent for other accessions (Figure 18).

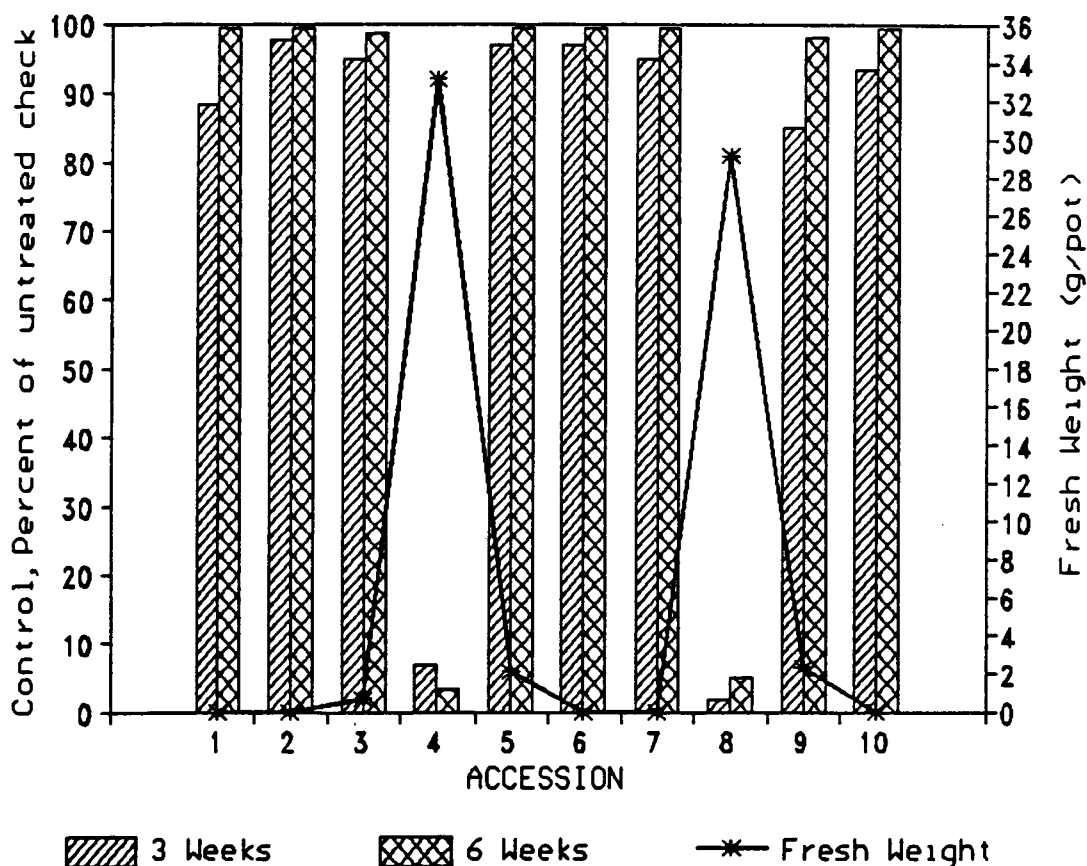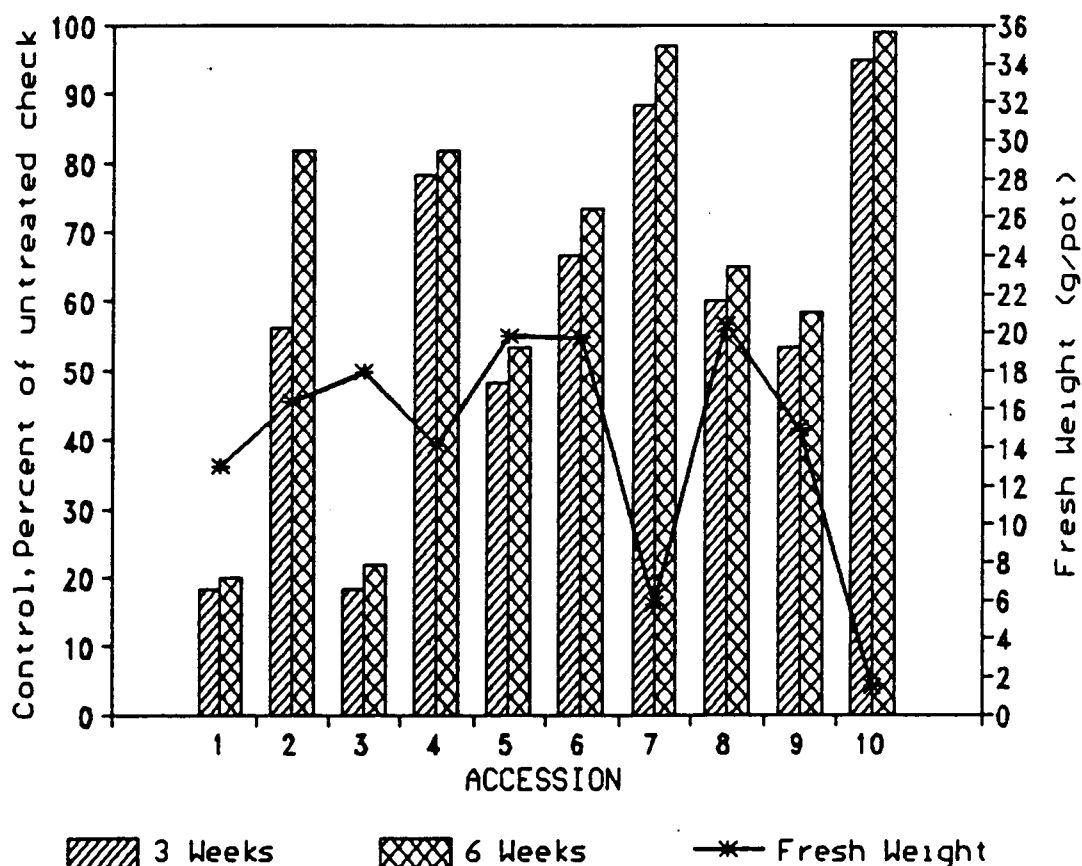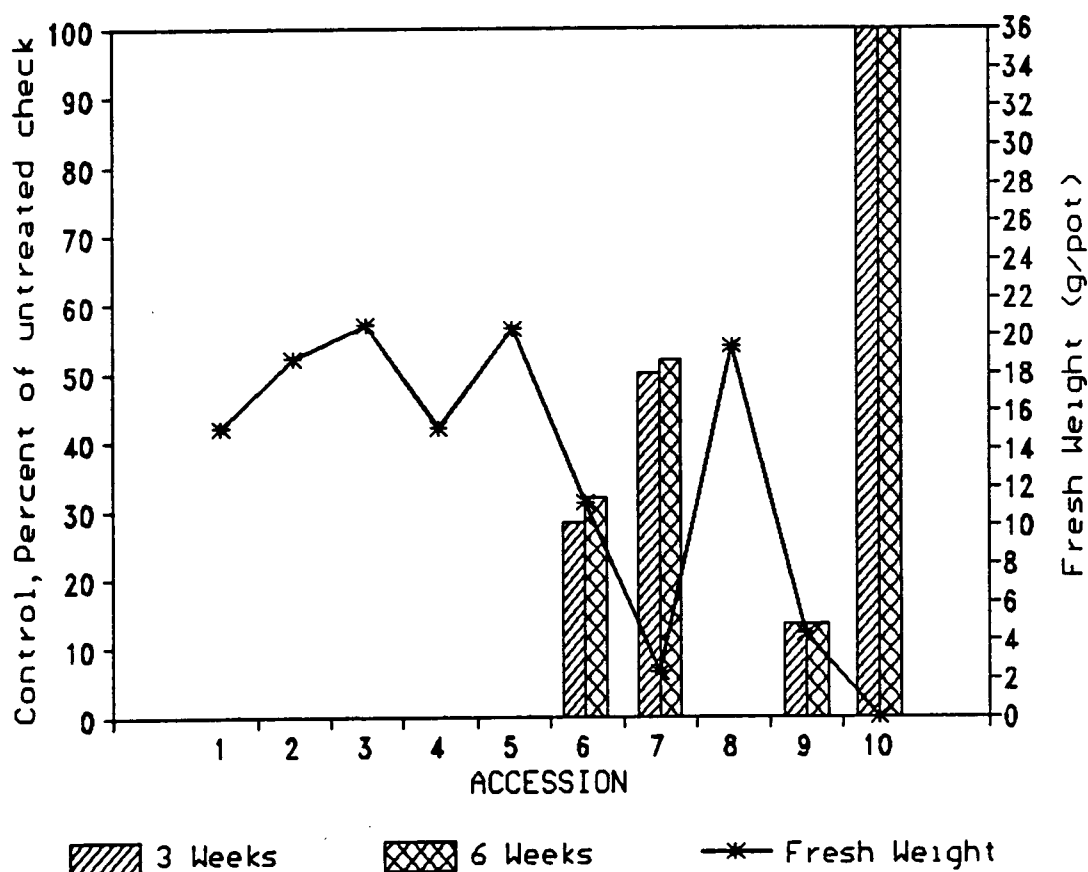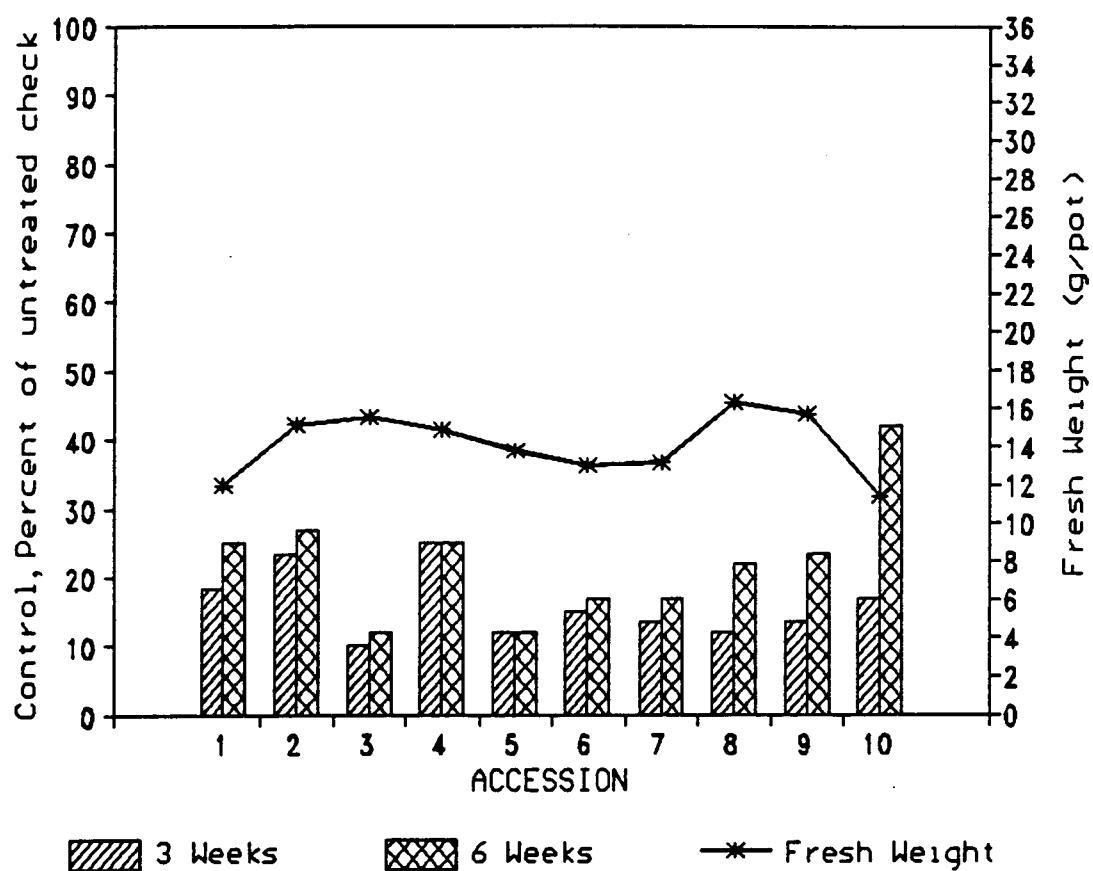Figure 18. Mean percent control at 3 and 6 WAT and fresh weight 6 WAT
of 10 accessions of Italian ryegrass (*L. multiflorum*) after
treatment with pronamide.
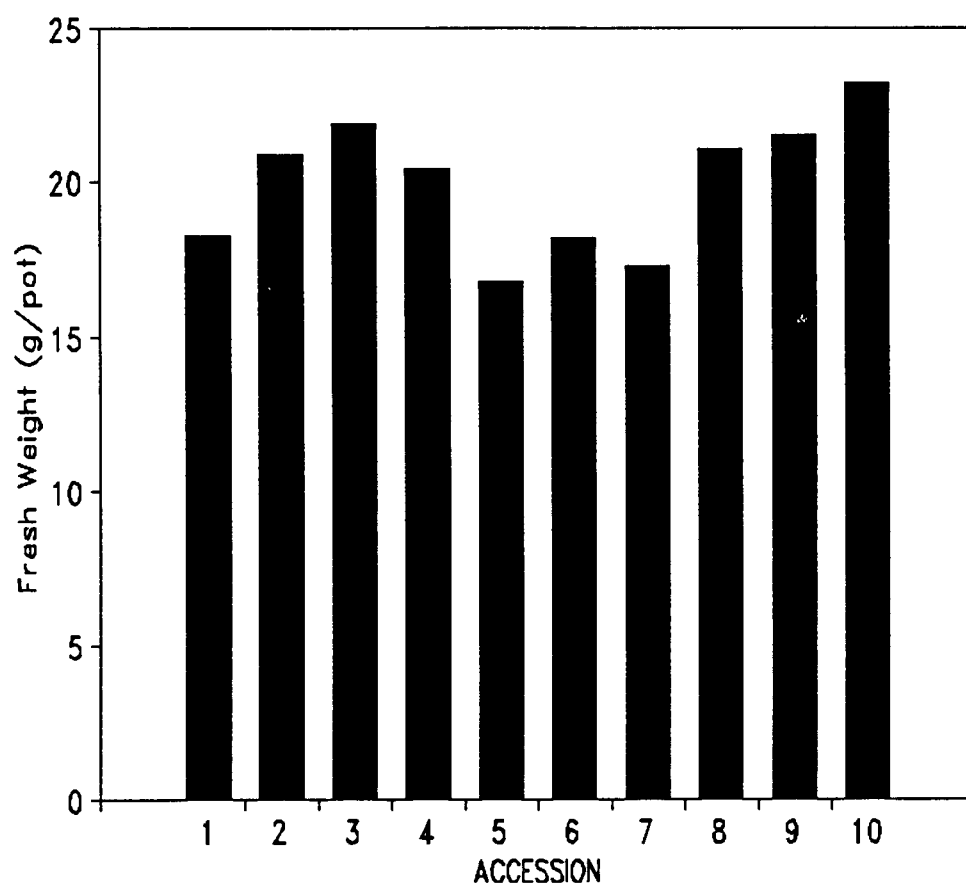
Figure 19. Fresh weight of accessions of Italian ryegrass (*L. multiflorum*) 6 weeks after planting, without herbicide application.

SUMMARY AND CONCLUSION

The purpose of this study was to determine the emergence and growth of nine accessions of Italian ryegrass which are said to have developed resistance to diclofop, and further to evaluate their cross or multiple resistance to other herbicides which have similar or dissimilar mode of action with diclofop.

Two of the diclofop-resistant accessions were resistant to diuron, but not to other herbicides. Percent emergence was lower for these resistant accessions than for the susceptible accessions except for accession 6.

The mechanism for resistance to diclofop is unknown. Resistance in plants may result from differential absorption or translocation of the herbicide or from detoxification. It is perhaps less likely to be differential absorption. Heap et al. (1986) noted that if resistance of rigid ryegrass was due to differential absorption, it would need to be effective for all the exposed tissues of their tested seed at germination and also of the shoot. Resistance is more likely the result of exclusion of diclofop-methyl from its site of action or from detoxification once inside the plant.

The development of resistance in accessions 4 and 8 to diuron would be hard to explain from the results of this experiment. However, it is suspected that these diuron-resistant populations developed in fields where diuron was applied for several years.

Herbicide resistance in general is now a widespread phenomenon throughout the world with numerous classes of herbicides. This increases the potential of increasing yield losses with a subsequent reduction in farming profitability. There is a crucial need, especially

in Third World countries, for governments, industry, the seed science community, and farmers to set up new priorities in future research. Present agronomic practices have to be examined to determine where mistakes have been made that have resulted in the development of resistance. Alternative and rational integrated weed management programs must be developed in order to meet the ever changing situations in the field with different cropping systems. Above all, farmers should realize that if they want to continue to rely on cost-effective and selective herbicides, they must be prepared to adopt changes and modify their farming and weed control practices continuously.

It is also important to note that resistant populations appear to have originated independently as a result of diclofop-methyl usage and differ in their levels of resistance to the herbicide as evidenced in this study. Ryegrass accessions resistant to diclofop-methyl may exhibit cross or multiple resistance to other herbicides (e.g. diuron) to which they might not necessarily have been exposed. Resistant populations also vary in their level of cross or multiple resistance. In view of the variations between accessions, farmers would be wise to test for cross or multiple resistance if they plan to continue to use diclofop and diuron to control Italian ryegrass.

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APPENDIX

FIELD EXPERIMENT

Table 1. Analysis of variance table for percent control at 3WAT of 9 accessions of diclofop-resistant Italian ryegrass (L. multiflorum L.) and a commercial population treated with post emergence herbicides (pronamide, diuron, diclofop-methyl and metribuzine).

Source	Degrees of freedom	Sum of Squares	Mean Squares	F	Pr>F
Replication	3	1031.06	343.7		0.0001
Herbicide	4	253676.7	63419.2	427.2	0.0001
Error (a)	12	1781.4	148.5		0.0003
Biotype	9	22985.1	2553.9	57.39	0.0001
Herbicide x Biotype	36	60067.2	1668.5	37.50	0.0001
Error (b)	135	6007.5	44.5		
Total	199	345549.0			

CV = 14.37%

Table 2. Analysis of variance table for percent control at 6WAT of 9 accessions of diclofop-resistant Italian ryegrass (L. multiflorum L.) and a commercial population treated with post emergence herbicides (pronamide, diuron, diclofop-methyl and metribuzine).

Source	Degrees of freedom	Sum of Squares	Mean Squares	F	Pr>F
Replication	3	579.5	193.2		
Herbicide	4	261156.9	65289.2	660.14	.0001
Error (a)	12	1186.8	98.9		
Biotype	9	24587.3	2731.9	67.21	.0001
Herbicide x Biotype	36	66660.3	1851.7	45.55	.0001
Error (b)	135	5487.5	40.6		
Total	199	359658.4			

CV = 12.72%

Table 3. Analysis of variance table for percent control at 9WAT of 9
          accessions of diclofop-resistant Italian ryegrass (L.
          multiflorum L.) and a commercial population treated with post
          emergence herbicides (pronamide, diuron, diclofop-methyl and
          metribuzine).

Source	Degrees of freedom	Sum of Squares	Mean Squares	F	Pr>F
Replication	3	562.2	187.4	5.24	
Herbicide	4	271579.9	67894.9	420.01	.0001
Error (a)	12	1939.8	161.7		
Biotype	9	24243.0	2693.7	75.37	.0001
Herbicide x Biotype	36	66566.1	1849.1	51.74	.0001
Error (b)	135	4824.8	35.7		
Total	199	369715.8			

CV = 11.45%

Table 4. Analysis of variance table for fresh weight at harvest of 9
          accessions of diclofop-resistant Italian ryegrass and a
          commercial population treated with post emergent herbicides
          (pronamide, diuron, diclofop-methyl and metribuzin).

Source	Degrees of freedom	Sum of Squares	Mean Squares	F	Pr>F
Replication	3	4795.4	1598.6		.0021
Herbicide	4	1316397.7	329099.4	193.15	
Error (a)	12	20446.1	1703.8		.0001
Biotype	9	67378.2	7486.5	23.14	.0001
Herbicide x Biotype	36	329210.8	9144.7	28.27	.0001
Error (b)	135	43669.0	323.5		
Total	199	1781897.4			

CV = 22.49

Table 5. Analysis of variance table for percent control at 3WAT of 9 accessions of diclofop-resistant Italian ryegrass (*L. multiflorum* L.) and a commercial population treated with preemergence herbicides (pronamide, UBI C4243, triallate, and trifluralin).

Source	Degrees of freedom	Sum of Squares	Mean Squares	F	Pr>F
Replication	3	26.7	8.89	.62	.6019
Herbicide	4	255752.3	63938.17	753.16	.0001
Error (a)	12	1018.7	84.89	5.94	.0001
Biotype	9	952.2	105.80	7.40	.0001
Herbicide x Biotype	36	3155.7	87.66	6.13	.0001
Error (b)	135	1930.1	14.30		
Total	199	262835.7			

CV = 5.37