

AN ABSTRACT OF THE THESIS OF

Maurice Philip Rolston for the degree of Doctor of Philosophy  
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Title: Control of Volunteer Legumes During Establishment of  
Legume Seed Crops With Activated Carbon Bands and Non-  
Selective Herbicides

Abstract Approved:

Redacted for privacy

Arnold P. Appleby

Redacted for privacy

W. Orvid Lee

In legume seed production, volunteer legumes arising from seed in the soil may result in intervarietal and interspecific contamination of seed lots. The survival of legume seeds in the soil is related to the presence of a water-impermeable palisade layer of macrosclerid cells in the testa. A detailed review of water-impermeable (hard) seed dormancy in Fabaceae (legumes) and species from other families including Convolvulaceae, Malvaceae, and Rhamnaceae is presented. The development of water-impermeable seed is controlled by genetic and environmental factors, especially relative humidity at seed maturation. The mechanism of natural softening in Papilionoideae (Faboideae) legumes is related to a weakening of the testa at the strophiole, especially by high or

fluctuating temperatures.

A possible method to control volunteer legumes is to apply activated carbon in a band over the seed row at the time of seeding the desired variety of legume. Subsequently, a blanket application of a non-selective herbicide, such as diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is made. The carbon adsorbs the herbicide, protecting the germinating seedlings below the band. Greenhouse and field experiments were conducted in western Oregon with white clover (Trifolium repens L.), red clover (T. pratense L.), and alfalfa (Medicago sativa L.) to determine (a) the tolerance of the three legumes as a crop, and (b) the control of volunteer legumes with carbon banding-herbicide treatments.

In the greenhouse, all three legumes could be protected with carbon from 4 kg/ha diuron. The order of tolerance to diuron was alfalfa > red clover > white clover. With white clover, the effective width of protection from a 25-mm carbon band was 13 mm with 4 kg/ha diuron. Carbon to herbicide ratios to protect white clover from diuron, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], and simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine] were 50:1, 200:1, 200:1, respectively.

In the field, white clover seedling counts were reduced to 64, 42, and 22% of the check by diuron at 2, 3, and 4 kg/ha, respectively. This resulted in seed yields at first harvest of 76, 44, and 12% of

the check. However, one year later, the seed yields from the same plots established with 2 and 3 kg/ha diuron were similar to the check. White clover injury due to diuron was greatest when herbicides were applied to soil that was wet on the surface. Alfalfa yields at first harvest were not significantly less than the check for plots established with diuron up to 4 kg/ha. Seed yields from red clover established with carbon bands and 0, 2, and 4 kg/ha diuron averaged, across all treatments, 566, 560, and 348 kg/ha. Seed germination and seed weights of white and red clover were not adversely reduced by diuron.

Good weed control between the carbon bands was obtained with the herbicides. However, grass and broadleaf weeds were also protected by the carbon band. In the greenhouse, granular EPTC (S-ethyl dipropylthiocarbamate) seeded with alfalfa at 10 mg/m of row controlled volunteer perennial ryegrass (Lolium perenne L.) without alfalfa injury. Using carbon banding with diuron, volunteer narrow birdsfoot trefoil (Lotus tenuis Waldst. and Kit.) was reduced by 98%. Volunteer white clover was reduced by 85 to 100% and volunteer red clover by 85 to 97% by diuron applied at rates up to 4 kg/ha. Loss of plants within the row due to competition reduced both the crop and volunteer population in the carbon band.

Control of Volunteer Legumes During Establishment  
of Legume Seed Crops with Activated Carbon  
Bands and Non-Selective Herbicides

by

M. Philip Rolston

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APPROVED:

Redacted for privacy

\_\_\_\_\_  
Professor of Crop Science  
in charge of major

Redacted for privacy

\_\_\_\_\_  
Courtesy Associate Professor of Crop Science

Redacted for privacy

\_\_\_\_\_  
Head of Department of Crop Science

Redacted for privacy

\_\_\_\_\_  
Dean of Graduate School

Date this thesis is presented November 27, 1978

Typed by Margi Wolski for M. Philip Rolston

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# CONTROL OF VOLUNTEER LEGUMES DURING ESTABLISHMENT OF LEGUME SEED CROPS WITH ACTIVATED CARBON AND NON-SELECTIVE HERBICIDES

## I. INTRODUCTION

In legume seed production, volunteer legumes arising from residual seed in the soil may result in contamination of seed lots. The survival of legume seeds in the soil is related to the presence of a water-impermeable palisade layer of macrosclerid cells in the testa. A review on water impermeable seed dormancy, prepared in a format for the Botanical Review, is presented in Chapter Two.

There are several possible approaches to reducing contamination of seed lots including (a) the reduction of hard seed in the soil by attempting to chemically and/or physically stimulate germination before the crop is planted and (b) to control volunteers as they germinate with the crop. The latter approach was selected for this thesis. There are no herbicides that will selectively control one variety of legume in another variety. However, non-selective herbicides can be made selective if the crop is protected from the herbicide by applying activated carbon in a band above the seed row. The activated carbon adsorbs the herbicide and thereby protects the germinating crop. The research undertaken for this thesis explored the possibility of using activated carbon with herbicides to establish legumes and control volunteer legumes. The results of this study, Chapters Three and Four, are prepared in a format for the Agronomy Journal.

## II. WATER IMPERMEABLE SEED DORMANCY

### Abstract

Viable seeds that do not imbibe water and thus fail to germinate in an apparently favorable environment are commonly termed impermeable or hard seed. This physical, exogenous dormancy is especially common in species of the Fabaceae. The ecological significance of hard seed includes the ability to rapidly recolonize burnt areas after fire and to withstand ingestion by animals and birds. Advantages and problems that hard seed cause in agriculture are discussed. Species from different families with impermeable seeds appear to have in common a layer of macrosclerid cells that form a palisade layer in the testa. The term strophiole and its contradictory use in botanical literature are discussed. Genetic factors and environmental conditions both affect the proportion of impermeable seeds produced. Methods of artificially softening impermeable seeds include acid and solvent soaking, mechanical scarification, pressure, percussion, freezing, heating, and radiation treatments that can result in a change in germination from less than 20% in some untreated species up to 90% or more in treated species. Natural softening involves high temperatures and temperature fluctuations and the degree of desiccation of the seed. The mechanism of water impermeability is related to the testa and is thought to involve waterproofing substances including wax, lignin, tannin, suberin, pectin, and quinone derivatives. The hilum acts as a hygroscopic valve that prevents water uptake but allows water loss to occur at low relative humidities in some species. The strophiole is an area of weakness in the testa of some Papilionoideae (Faboideae) while the chalaza region has been determined as an area of weakness in Pisum and Gossypium. The

water impermeable status of some species is reversible at a seed moisture content greater than 10%. The hard seed of a species can be described both in terms of the amount and the degree of impermeability.

### Introduction

Viable seeds sometimes will not imbibe water and therefore fail to germinate, even when conditions are apparently favorable for germination. Seeds that are unable to imbibe water are commonly termed impermeable or hard seed; they remain hard compared to imbibed seeds which soften during germination. Impermeable seeds are common in many species of Fabaceae (legumes) as well as occurring in some species of Cannaceae, Chenopodiaceae, Convallariaceae, Convolvulaceae, Geraniaceae, Malvaceae, Solanaceae (Harrington, 1916), Anacardiaceae (Stone and Juhren, 1951), and Rhamnaceae (Gratkowski, 1962). Of 260 legume species examined, 85% had some or all impermeable seeds (Guppy, 1912).

The water impermeability of the testa of hard seed is a physical exogenous dormancy (Nikolaeva, 1969), which may or may not be combined with other dormancy mechanisms. Examples of combined dormancy include cold stratification responses in Cercis canadensis (Afanasiev, 1944) and scarified linden (Nikolaeva, 1969); after-ripening during storage of red clover (Trifolium pratense) and subterranean clover (T. subterraneum) reported by Martin (1944) and Woodforde (1935); and responses to high CO<sub>2</sub> concentrations by scarified subterranean clover (Grant Lipp and Ballard, 1959).

Information on water impermeable seeds has been cataloged by Barton (1967). Reviews and textbooks often give only brief attention to the dormancy of impermeable seeds (Crocker, 1948; Crocker and

Barton, 1953; Eames, 1961; Salisbury and Ross, 1969; Nikolaeva, 1969; Villiers, 1972; Mayer and Poljakoff-Mayber, 1975; Copeland, 1976) while detailed reviews on seed impermeability have been presented by Porter (1949), Barton (1965), Quinlivan (1971a), and Ballard (1973). Although it is just over 100 years since Nobbe (1876) introduced the term "hard seed," our knowledge of the anatomy and physiology of this process is still incomplete.

The purpose of this review is to (a) incorporate recent additions and unpublished theses into the literature; (b) discuss aspects of water impermeable seed dormancy not presented in detail before, including the comparative anatomy of hard seed in several families, inheritance of hard seed characteristics and the use of the word "strophiole" in botanical literature; and (c) discuss factors that cause softening of hard seeds.

### Ecological and Agricultural Significance

Impermeable seed coats permit extension of life to many seeds so that they are distributed in time as well as in space (Crocker and Barton, 1953). Not only do impermeable seeds remain viable for a long time, but under natural conditions increments of a seed population become permeable to water and germinate in successive intervals (Williams and Elliott, 1960). The impermeable seeds of Acacia melanoxylon (Villiers, 1972), Ceanothus sp. (Gratkowski, 1962, 1973), Rhus ovata (Stone and Juhren, 1951), and Ulex europaeus (Rolston, unpublished data) will germinate following fire, allowing rapid recolonization of the burned area. The ability of impermeable seeds to survive ingestion (Table 1) allows dispersal by animals and birds (Pammel, 1899; Harmon and Keim, 1934; Burton and Andrews, 1948) while Suckling (1950, 1952) has utilized sheep to disseminate white

TABLE 1. Recovery of impermeable seeds fed to livestock adapted from Harmon and Keim (1934).<sup>1,2</sup>

Species	% Recovery	Germination %			
		Non-acid		Acid Treated	
		Before	After	Before	After
<u>Convolvulus arvensis</u>	23	9.3	10.4	84.0	54.0
<u>Melilotus alba</u>	15	7.8	14.0	69.5	68.0

<sup>1</sup> Average of seeds fed to calves, horses, sheep, hogs, chickens.

<sup>2</sup> Adapted from Journal of the American Society of Agronomy, Volume 26, page 765, 1934 by permission of the American Society of Agronomy.

clover seed in hill country grazing lands. Seed longevity is often, but not always, associated with impermeable seed (Quick, 1961; Quinlivan, 1971a; Harrington, 1972).

Impermeable seeds have a number of advantages in agriculture. The maintenance of cotton (Gossypium hirsutum) seed quality under high humidity storage conditions (Christiansen et al., 1960) and the prevention of preharvest deterioration in damp seasons (Christiansen and Justus, 1963) is correlated with hard seed characteristics. Re-seeding in annual legumes is a desirable characteristic and is related to the hard seed characteristic in Vicia (Donnelly and Clark, 1962) and crimson clover (Trifolium incarnatum) (Hollowell and Knight, 1962). Bennett (1959) believed that germination at a moisture level too low for subsequent seedling establishment of crimson clover is the predominant cause of unsatisfactory stands. A degree of hard seededness is, therefore, required to help delay germination of some

seeds until adequate moisture occurs.

In perennial crops, impermeable seed may be considered as a potential reserve of the species, to make up for empty spots in the stand or to help in establishing the stand following unfavorable conditions, while Dexter (1955) found that impermeable seeds of alfalfa (Medicago sativa) provided considerable insurance against late frosts and other field hazards. However, Harrington (1916) suggested that because of the slow germination and development of plants from hard seeds, they cannot compete with established plants. In annual crops, a wide range in time of emergence, delayed harvest, and poor yield (Baciu-Miclaus, 1970) or an inability to compete successfully with older plants and weeds are disadvantages of hard seed (Nelson et al., 1964).

A number of weed species, including field bindweed (Convolvulus arvensis), velvetleaf (Abutilon theophrasti), and Ulex europaeus, produce impermeable seeds and will continue to give problems long after flowering parent plants are controlled. Even some crop plants, especially the forage legumes, have become weeds in certain situations due, in part, to the persistence of impermeable seeds in the soil. Intra and interspecific contamination of forage legumes can be a problem in seed production (Shillito, 1974).

In food processing, hard seeds in beans are considered undesirable by bean canners who want seed lines free of hard seed, yet relatively low in moisture. To meet this latter requirement, beans were often artificially dried, which tended to increase the hard seed content (Lebedeff, 1947).

## Factors Affecting Seed Impermeability

### A. Anatomy of the Testa

The testa of species with water impermeable seeds from four families have been studied, *Convulvulaceae* (Sripleng and Smith, 1960; Callihan, 1961), *Fabaceae* (Pammel, 1899; Watson, 1948; Corner, 1951, 1976; Scott et al., 1962; Chowdhury and Buth, 1970; Vaughan, 1970), *Malvaceae* (Reaves and Valle, 1932; Simpson et al., 1940; Leahy, 1948; Winter, 1960), and *Rhamnaceae* (Gratkowski, 1962). The testa may be the result of differentiation of only the outer integument as in the legume ovule (Esau, 1960) or of the single integument of the field bindweed ovule (Sripleng and Smith, 1960).

1. The Outer Integument. A common feature of water impermeable seeds is a palisade layer of macrosclerid cells (Fig. 1), also called epidermal, prism, or malpighian cells. The outer end of these cells is pointed or domed and has a characteristic thickening of the cell wall termed a terminal cap. In the pea (*Pisum sativum*) and in *Tetragonolobus conjungatus* and *T. requienii*, there are specific suberin adcrustations situated on the outer ends of the macrosclerid cell wall; however, these are not present in other species of legumes examined (Spurny, 1963, 1972).

Through the palisade layer of macrosclerids, one (or sometimes two) light lines, the linea lucida of earlier papers (Pammel, 1899), may be observed. These light lines result from the difference in the refraction of light due to a change in the chemical composition of the macrosclerid. In cotton there is a greater proportion of lignin in the macrosclerid above the light line than adjoining areas below the light line (Reeves and Valle, 1932), while in *Melilotus*, *Medicago* and



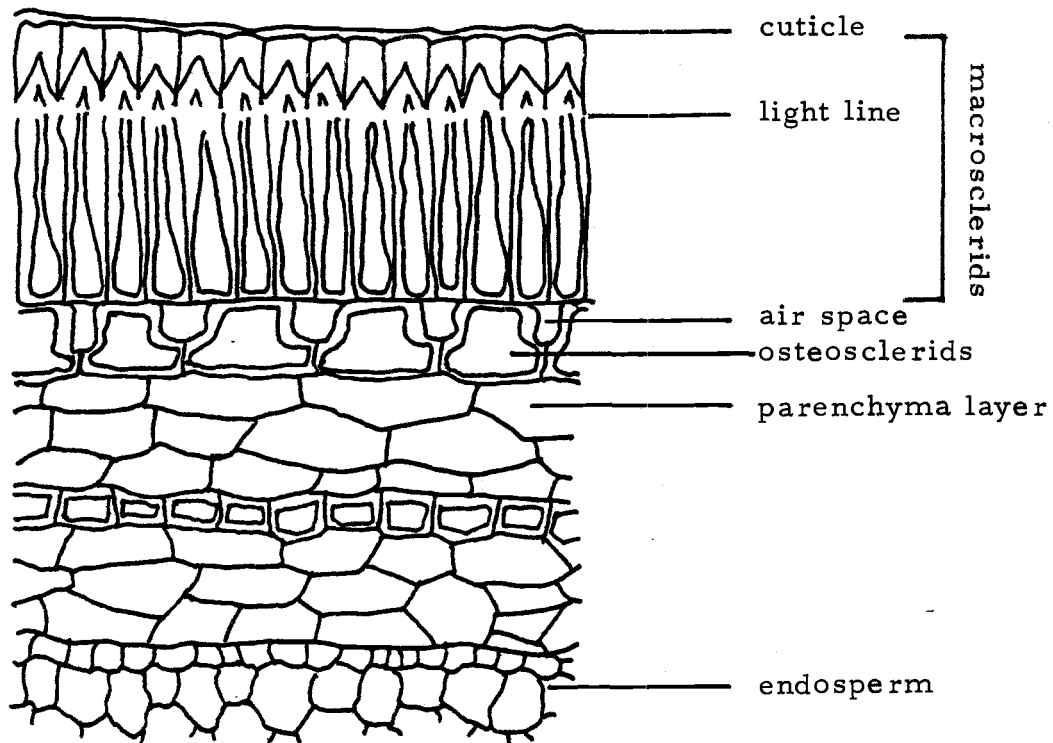


Fig. 1. Longitudinal section of the seed coat of sweet clover from Martin and Watt (1944).

Trifolium, the light line results from the juxtaposition of the suberin or cutin caps and the cellulose in the lower portion of the macro-sclerid (Hamly, 1932; Spurny, 1972).

External to the macrosclerid cells of cotton and field bindweed are two layers of cells. In cotton, the outer layer consists of epidermal cells from which the lint hairs arise, and below the outer epidermal cells lies the outer pigment cells (Leahy, 1948). In field bindweed, the outer layer is a pigmented epidermis of thick-walled elongated cells. The subepidermal cells are compact cubical sclerids, thick-walled, and lightly impregnated with lignin, cutin, and suberin (Sripleng and Smith, 1960). In legumes, the outer surface of the testa is immediately external to the macrosclerid cells and consists of a cuticle (Corner, 1951) or cuticle and subcuticle (Hamly, 1932) of variable thickness.

Interior to the macrosclerids of a legume is a subepidermal layer of osteosclerids, also called columnar, hourglass, pillar, or spool cells, depending on the distribution of wall thickenings and shape of cells. Large intercellular spaces may occur between these cells (Esau, 1960).

Beneath the osteosclerids in legumes, a parenchyma layer of up to 12 cells thick (Pammel, 1899) has been variously described as being composed of nutrient, collapsed nutrient, parenchyma, crushed parenchyma, mesophyll or spony mesophyll cells. In field bindweed, the layer internal to the macrosclerid cells consists of parenchyma cells that are crushed at maturity (Sripleng and Smith, 1960). Beneath the parenchyma layer lies the cells of the endosperm.

2. Hilum. The hilum (scar left by the stalk of the ovary) of the Papilionoideae legumes (Hyde, 1954) and field bindweed (Callihan, 1961) consist of both palisade and counter-palisade layers of macrosclerids (Fig. 2a). Legumes in the subfamilies Mimosoideae and Caesalipinioideae have a hilum closed by a single palisade or without palisade macrosclerid cells (Corner, 1951). The hilum of Ceanothus velutinus (Rhamnaceae) also has a single palisade layer (Gratkowski, 1962). A chalazal discontinuity of the palisade layer occurs in velvet-leaf and cotton (Fig. 2b) where the vascular tissue from the ovary penetrates the seed. This discontinuity is described as a chalazal slit,  $60 \times 740\mu$  in velvetleaf, while in cotton it is a pore (chalazal aperture) 240 to  $280\mu$  in diameter (Winter, 1960) with a plug of dense parenchyma tissue (Leahy, 1948).

3. Strophiole. A structural feature commonly mentioned in literature on hard seeds is the strophiole. Unfortunately, the word "strophiole" has been used with two different meanings in seed

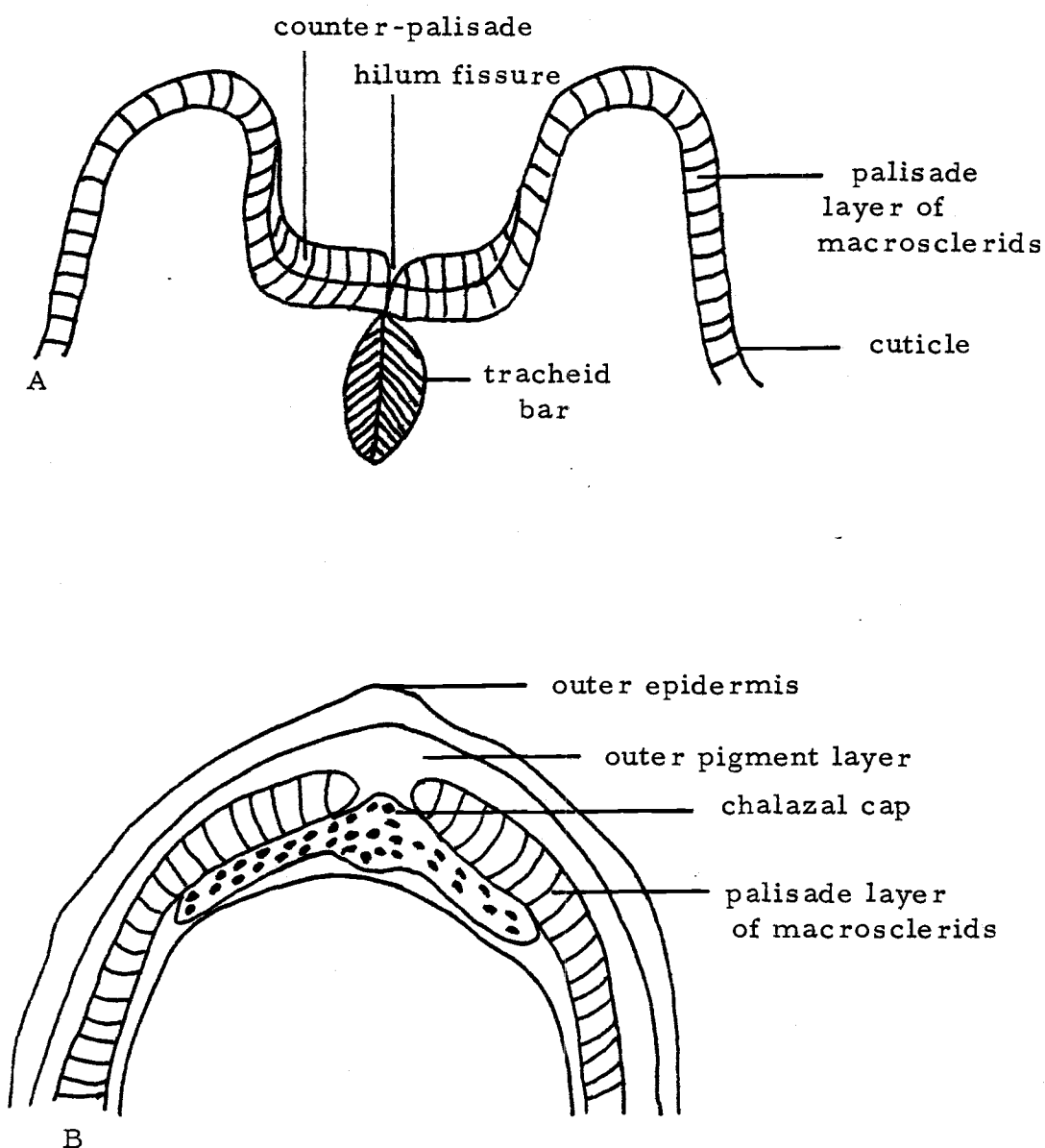


Fig. 2. Cross-section views of two types impermeable seeds.  
 (A) Hilum view of typical Papilionoideae legume adapted from Hyde (1954).  
 (B) Chalazal region of cotton adapted from Leahy (1948).

anatomy and the correct usage of the word is at present unresolved. One use of strophiole is in connection with ecological modifications related to seed dissemination, while a second use is to describe a structural modification of the palisade layer of the macrosclerid cells in some legume species.

In 1899, Rusby and Jelliffe described the strophiole as an external enlargement on the raphe that may develop into an aril. Eames (1961) described the aril as a proliferation or fleshy transformation of parts of the outer integument, chalaza, or distal part of the funicle. An aril restricted to crests on the seed, such as the raphe, is a strophiole and is a modification related to seed dissemination. Bhatnager and Johri (1972) and Fahn and Werker (1972) both describe strophioles as being either synonymous with, or a type of, elaiosome (an aril that is adapted to dispersal by ants).

In describing the strophiole as a structural feature of the palisade layer of macrosclerid cells, Hamly (1932) cites the work of Mattiolo and Buscalioni (1892). On the basis of this priority, a structural use of the term strophiole should be considered correct until evidence for the use of the word in any other context is found to predate the work of Mattiolo and Buscalioni (1892). The strophiole described by Hamly appears to be one characteristic of the legume subfamily Papilionoideae (Barton, 1947). He describes the strophiole of Melilotus alba as being centered in a narrow longitudinal depression, close to the hilum on the side opposite the micropyle. A diagram by Hutton and Porter (1937) shows a similar relationship in Amorpha fruticosa (Fig. 3). Hagon and Ballard (1970) describe the strophiole as a predetermined site of weakness in the testa through which imbibition can occur. In Trifolium subterraneum, they described the strophiole as being a swelling of the testa, on the raphe, between the hilum and chalaza.

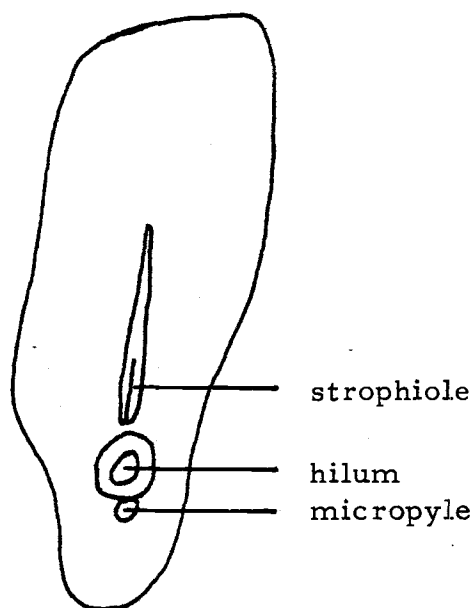


Fig. 3. Strophiole in relation to hilum and micropyle in Amorpha fruticosa from Hutton and Porter (1937).

In cross-sectional photographs and diagrams, the strophioles described by Hamly (1932), Aitken (1939), Ueki and Suetsugu (1958), and Hagon and Ballard (1970) appear similar in structure, consisting of an area where the macrosclerid cells are longer and narrower than normal, and on both sides short macrosclerid cells are found overlying loosely arranged cells (Fig. 4a). In subterranean clover, the macrosclerids of the strophiole are 120  $\mu\text{m}$  long and 6 to 8  $\mu\text{m}$  wide, while the macrosclerids remote from the strophiole are 34 to 40  $\mu\text{m}$  long and 10  $\mu\text{m}$  wide (Aitken, 1939; Hagon, 1972). The strophiolar cleft resulting from percussion (shaking) is due to the macrosclerid cells having pulled apart, forming a split, fissure or cleft between two of the adjacent macrosclerids (Fig. 4b). The strophiole of Astragalus sinicus is transparent in hard seed and opaque in soft seed. The change in transparency occurs when the strophiole splits, resulting in a physical change in the tissues (Ueki and Suetsugu, 1958).

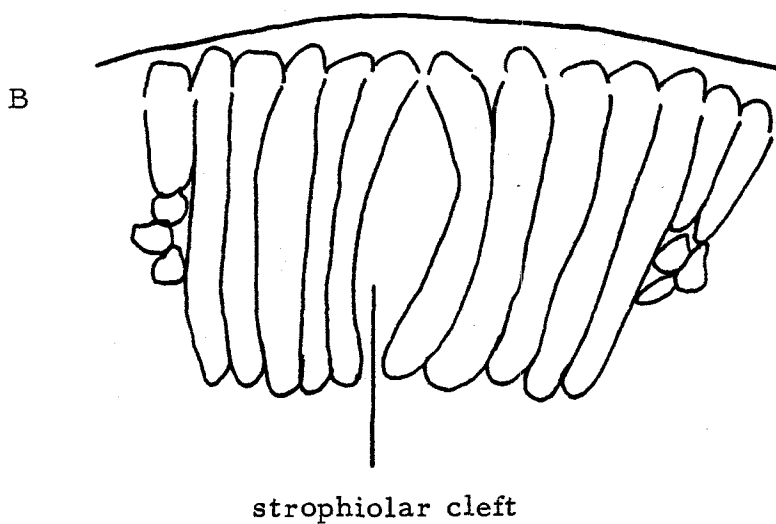
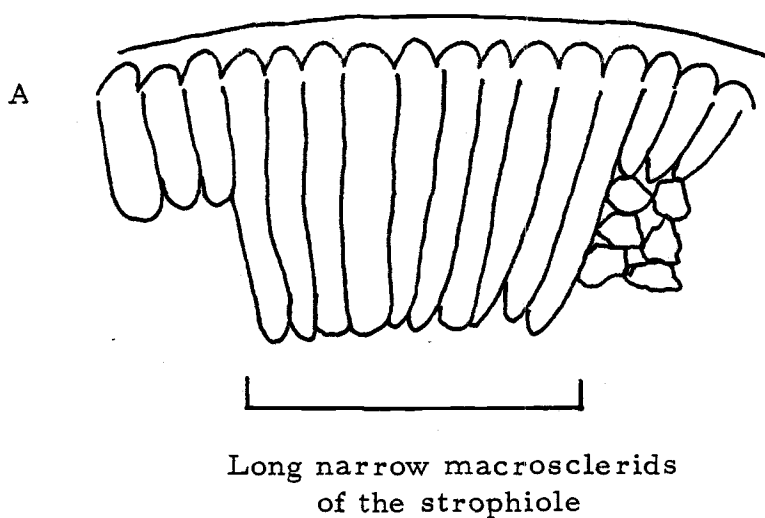


Fig. 4. Diagrammatic cross-sectional view of (A) intact strophiole and (B) percussed strophiole with strophiolar cleft penetrating the light line but not the cuticle. Adapted from Hamly (1932), by permission of the University of Chicago Press and Hagon and Ballard (1970).

Salisbury and Ross (1969) and Meyer and Poljakoff-Mayber (1975) suggest water entry is blocked by a cork-like material, the strophiolar plug, in a small opening, the strophiolar cleft. Only if this is loosened by impaction can water enter the seed. However, the "plug" concept is not consistent with the interpretation by Hamly (1932) or Hagon and Ballard (1970).

Another interpretation of the strophiole in Melilotus alba is that it is a hilum structure which is traversed at its outer edge by the vascular connections between the seed and the funiculus (Martin and Watt, 1944). This does not appear to be the same structure described by Hamly (1932).

## B. Environment

1. Relative Humidity and Temperature. Large variations occur in the impermeability of seed of the same species harvested in different localities or years (Table 2) (Loftus Hills, 1944; Dexter, 1947; Baciú-Miclaus, 1970). In alfalfa, seed from lower altitudes tended to

TABLE 2. Variations in the percentage impermeable seed for soybeans grown at different locations in two consecutive years, adapted from Baciú-Miclaus (1970).

Variety	Year	Location 1	Location 2	Location 3
Mandarin	1	59	34	9
	2	28	12	4
Lincoln	1	32	25	3
	2	15	13	-

be lower in hard seed content than seed from higher altitudes. Second or late harvested seed seemed to be somewhat higher in hard seed than first cutting or early harvested seed (Dexter, 1955). In soybeans, sites with low relative humidity (RH) and high temperatures developed more hard seed. Quinlivan (1971a) reviews the effect of RH on impermeability and its relationship to seed moisture content. Impermeable seed content and seed softening are a function of seed moisture. Two studies with beans (Phaseolus vulgaris) demonstrate this. Lebedeff (1947) exposed beans to 10 moisture levels, giving seed moisture contents ranging from 14.11% to 5.39%. The 14.11% moisture content beans had 1% impermeable seeds compared to 90% impermeable seeds for the 5.39% moisture content. Each reduction in the moisture content increased the percentage of impermeable seeds and the seeds remained hard for a longer period of time. Harrington (1949) found that the moisture content of the seed was determined by the RH and the time of exposure and the hard seed content increased as seed moisture decreased. These results in Papilionoideae legumes can be explained by the hilum acting as a hygroscopic valve (Hyde, 1954).

Semi-mature soft seeds of barrel medic (Medicago truncatula) that were buried 2 cm below the soil surface in two soil types, had less than 17% impermeable seeds 27 days later, compared to 97% for pods left on the surface (Kitchner and Andrew, 1971). Unfortunately, the temperature and moisture conditions in the soil and at the surface were not recorded.

2. Light. The effect of light on impermeable seed needs further investigation, following the report that reducing daylength during the last eight days of ripening increased the water permeability of Ononis sicula, a facultative, long-day winter annual legume



(Guttermann and Heydecker, 1973). A reduction in light intensity was without effect on seed impermeability in Trifolium incarnatum (James, 1949a).

3. Soil Fertility. Several experiments show small changes in the impermeable seed content of species treated with fertilizer. James (1949a) and James and Bancroft (1951) increased the hard seed content of crimson clover with  $\text{CaCO}_3$ , while potash (110 kg/ha, K) reduced impermeable seed content. el Bagoury and Niyazi (1973) recorded a depression in impermeable seeds of Trifolium alexandrinum with a combination of potassium sulphate (476 kg/ha) and ammonium sulphate (476 kg/ha) but no effect from the potassium sulphate alone. In a later experiment, el Bagoury (1975) increased the impermeable seed content of Vicia faba from 10% to 20% with potassium sulphate (238 kg/ha). Soil fertility appears to play a minor role in the development of impermeable seeds.

### C. Genetics

Hard seed in leguminous plants has been attributed to both genetic and environmental factors (Donnelly, 1970). Early attempts (Loftus Hills, 1944; James, 1949b) to demonstrate a genetic component in hard seeds were largely unsuccessful and it was concluded that if heritable factors were present, they were masked by environmental factors. Evidence for a genetic component has been established for a number of species. Using mass screening techniques, Bennett (1959) rapidly increased the impermeable seed content of crimson clover, while Donnelly (1971) produced an increase in the frequency of plants that produce 70-100% hard seed in successive generations of Vicia interspecific hybrids. Many of the Vicia sativa type  $F_6$  lines were

genetically stable for a high percentage of hard seed. Baciú-Miclaus (1970) reported varietal differences in the capacity of soybeans to develop impermeable seeds. In alfalfa, the hybrid Medicago media had an impermeable seed content (20%) compared to the parent types, M. sativa and M. falcata, which had 5% and 56% impermeable seeds, respectively (Mijatovic, 1971). Both the amount of hard seed and the rates of softening of the seed coat of impermeable bean seeds are inherited (Lebedeff, 1947). Relatively few genes appear to be involved in hard seed inheritance. Lebedeff (1947) concluded that only a few genes are involved in the differentiation of hard- and soft-seeded selection of Phaseolus vulgaris. Impermeable seed in blue lupine (Lupinus angustifolius) is controlled by a single dominant gene, whereas soft-seededness in two cultivated varieties was controlled by an allelic recessive pair (Forbes and Wells, 1968). In Vicia, Donnelly et al. (1972) report two gene inheritance, where gene A is simple dominant for hard seed and gene B is dominant for soft seed when the A locus is a homozygous recessive aa. Gladstones (1970), using Lupinus luteus, L. albus and L. angustifolius, established that impermeability was a simple dominant over permeability. A single allelic pair accounted for most of the expression of seed impermeability in an experimental line of cotton, with evidence for a second allelic pair with lesser effects (Lee, 1975).

A knowledge of the inheritance of hard seed will aid plant breeders in making appropriate crosses to increase or decrease the water impermeable content, depending on the objectives of the breeding program.

## Softening of Hard Seeds

### A. Artificial Softening

A variety of treatments that will induce hard seeds to germinate has been known for many years and the early methods have been reviewed by Porter (1949). Table 3 lists examples of various treatments on the germination of impermeable seeds.

1. Chemicals. Concentrated sulfuric acid has been used experimentally for many years (Hiltner, 1902; Hopkins, 1923) with considerable success on many species. Even apparently acid resistant species such as Lupinus cosentini become permeable after 4 to 7 hours soaking in concentrated sulfuric acid (Horn and Hill, 1974). Alcohol and other organic solvents reduced hard seed in Nelumbo lutea (Shaw, 1929) but results in other species have been variable (Cavazza, 1951). In a detailed study of the legume subfamilies, Barton (1947) found that seeds of species in Caesalpinioideae were made permeable by absolute ethyl alcohol, while those in Mimosoideae and Papilionoideae were generally unaffected or showed only slight increases in permeability. Acetone and petroleum ether caused small but significant reductions in the hard seed of crown vetch (Coronilla varia), while the membrane penetrant dimethyl sulfoxide (DSMO) in combination with water or ethyl alcohol had little effect (Brant et al., 1971). The seeds of field bindweed were made permeable by absolute ethyl alcohol but not by diluted alcohol (Callihan, 1961). Addition of two wetting agents to water had no effect in reducing the percentage of impermeable seed of crown vetch (Brant et al., 1971).

TABLE 3. Examples of the effects of different treatments on various impermeable seeds.

Treatment	Species	% Permeable		Author	Date
		Untreated	Treated		
ACID					
H <sub>2</sub> SO <sub>4</sub> (conc), 15 min.	<u>Centrosema pubescens</u>	28	88	Win Pe et al.	1975
H <sub>2</sub> SO <sub>4</sub> (conc), 30 min.	<u>Cercis canadensis</u>	10	96	Afanasiev	1944
H <sub>2</sub> SO <sub>4</sub> (conc), 30 min.	<u>Colutea istria</u>	5	98	Koller & Negbi	1955
H <sub>2</sub> SO <sub>4</sub> (conc), 30 min.	<u>Convolvulus lanatus</u>	0	84	Koller & Cohen	1959
H <sub>2</sub> SO <sub>4</sub> (18N), 30 min.	<u>Coronilla varia</u>	29	84	Brant et al.	1971
H <sub>2</sub> SO <sub>4</sub> (conc), 3 hr.	<u>Rhus ovata</u>	3	62	Stone & Juhren	1951
H <sub>2</sub> SO <sub>4</sub> (conc), 30 min.	<u>Strophostyles helvola</u>	29	79	Hutton & Porter	1937
SOLVENTS					
Absolute ethyl alcohol, 82 hr.	<u>Acacia constricta</u>	9	88	Barton	1947
Absolute ethyl alcohol, 20 hr.	<u>Convolvulus arvensis</u>	15	90	Callihan	1961
Absolute ethyl alcohol, 72 hr.	<u>Gymnocladus dioica</u>	4	92	Barton	1947
PRESSURE					
2000 atm, 10 min.	<u>Melilotus alba</u>	24	83	Davis	1928
2000 atm, 1 min.	<u>Medicago sativa</u>	48	83	Davis	1928
PERCUSSION					
Shaken, 10 min.	<u>Amorpha fruticosa</u>	46	73	Hutton & Porter	1937
Shaken, 20 min.	<u>Cladrastis lutea</u>	6	82	Barton	1947
Shaken, 6 hr.	<u>Colutea istria</u>	5	90	Koller & Negbi	1955

(Continued)

TABLE 3. Continued.

Treatment	Species	% Permeable		Author	Date
		Untreated	Treated		
Shaken, 4 hr.	<u>Convolvulus lanatus</u>	0	94	Koller & Cohen	1959
Shaken, 10 min.	<u>Lespedeza capitata</u>	4	84	Hutton & Porter	1937
Shaken, 5 min., 550 oscill/min.	<u>Trifolium subterraneum</u>	12	63	Ballard & Grant Lipp	1965
<u>SCARIFICATION</u>					
Mechanical	<u>Astragalus cicer</u>	42	93	Townsend & McGinnies	1972
Mechanical	<u>Centrosema pubescens</u>	28	85	Win Pe et al.	1975
Filed	<u>Convolvulus arvensis</u>	6	98	Callihan	1961
Grindstone	<u>Crotalaria occaleuca</u>	10	58	Jones	1971
Mechanical	<u>Desmodium uncinatum</u>	2	56	Keya & van Eijnatten	1975
Hole drilled through seed coat	<u>Rhus ovata</u>	3	74	Stone & Juhren	1951
Abraded	<u>Trifolium hybridum</u>	4	81	Nakamura	1962
Abraded	<u>Trifolium repens</u>	11	51	Nakamura	1962
<u>FREEZING</u>					
-195.8°C	<u>Coronilla varia</u>	16	68	Brant et al.	1971
-15°C, 1 hr.	<u>Medicago sativa</u>	5	21	Midgley	1926
<u>HEATING</u>					
Water, 70°C, 30 sec.	<u>Abutilon theophrasti</u>	20	98	LaCroix & Staniforth	1964
95°C, 6 min.	<u>Abutilon theophrasti</u>	34	85	LaCroix & Staniforth	1964
(Continued)					

TABLE 3. Continued.

Treatment	Species	% Permeable		Author	Date
		Untreated	Treated		
Water, 90°C	<u>Ceanothus sanguineus</u>	5	90	Gratkowski	1973
Boiling water, 30 sec.	<u>Coronilla varia</u>	17	49	Brant et al.	1971
510°C, contact heat	<u>Medicago sativa</u>	34	78	Lunden & Kinch	1957
60°C, 20 min.	<u>Medicago sativa</u>	37	68	Stetson & Nelson	1972
100°C, 5 min.	<u>Rhus ovata</u>	3	34	Stone & Juhren	1951
<u>RADIATION</u>					
Glow discharge, 3 min.	<u>Gossypium hirsutum</u>	38	73	Goodenough et al.	1970
Glow discharge, 60 Hz, 3 min.	<u>Gossypium hirsutum</u>	3	58	Stone et al.	1973
IR, 1.1 sec.	<u>Medicago sativa</u>	48	83	Nelson et al.	1968
RF, 39 MHz, 5.8 sec.	<u>Medicago sativa</u>	48	88	Nelson et al.	1968
Microwave 2450 MHz, 80 sec.	<u>Medicago sativa</u>	32	75	Stetson & Nelson	1972
<u>SEED STORAGE</u>					
Drying, over CaCl <sub>2</sub>	<u>Abutilon theophrasti</u>	12	72	LaCroix & Staniforth	1964
Moist cabinets, 20 months	<u>Medicago truncatula</u>	2	48	Kirchner & Andrew	1971

2. Enzymes. Hemicellulase and pectinase reduced the impermeable seed of crown vetch to 48% and 54%, respectively, compared to the controls containing 68% impermeable seed (Brant et al., 1971).

3. Pressure. High pressures (500 to 2000 atm.) reduce the occurrence of impermeable seeds in a number of species (Davis, 1928a, b; Rivera et al., 1937).

4. Percussion. Percussion, referred to as impaction in early papers (Hamly, 1932), resulting from vigorous shaking reduces seed impermeability (Hamly, 1932; Hutton and Porter, 1937). Legume species sensitive to 20 minutes shaking (mostly members of the Papilionoideae and several Mimosoideae species) were generally resistant to ethyl alcohol treatments and vice versa, with one exception, Parkinsonia microphylla (Caesalpinioideae), which became permeable with both treatments.

5. Scarification. Mechanical scarification, developed by Hughes (1915) utilizes abrasion, particularly by rough surfaces, and is probably the most common commercial treatment for impermeable seeds. Machine harvesting markedly reduces the impermeable seed content; e.g., in Trifolium hirtum, hand-harvested and machine-harvested samples had 86% and 30% impermeable seed, respectively (Ballard et al., 1976). Injury during scarification, resulting in decreased vigor and viability (Hamly, 1932) depends on the method of scarification (Jones, 1971). Repeated scarification in cicer milk-vetch (Astragalus cicer) gave germinations of 80% or greater but was associated with poor field establishment (Carleton et al., 1971).

6. Freezing. Freezing but not subsequent freezing and thawing reduced the amount of impermeable alfalfa seed (Midgley, 1926). The use of liquid air ( $-190^{\circ}\text{C}$ ), liquid nitrogen ( $-195.8^{\circ}\text{C}$ ), and liquid oxygen ( $-185^{\circ}\text{C}$ ) have been successfully used to reduce impermeable seed in a number of legume species (Busse, 1930; Barton, 1947; Enyard, 1957, 1958; Brant et al., 1971).

7. Heating. Both hot water (Thornber, 1904; Rodriquez, 1924) and dry heat (Stewart, 1926; Lute, 1928; Rincker, 1954; Lunden and Kinch, 1957) have been used successfully to reduce impermeable seed content. Moderate dry heating ( $41^{\circ}\text{C}$ ) for five days markedly increased germination and reduced the number of impermeable alfalfa seeds (Ellis and Palmer, 1973), while in crown vetch  $100^{\circ}\text{C}$  and  $125^{\circ}\text{C}$  for 1 to 24 hours reduced the impermeable seed content, but resulted in nearly complete death of the seed (Brant et al., 1971).

8. Radiation. Considerable interest has developed in the commercial feasibility of using electrically generated radiations as an effective alternative to scarification. Rincker (1954) reported that infrared radiation (IR) reduced the impermeable seed content of alfalfa and red clover but not sweet clover, and Eglitis and Johnson (1957) found similar results with high frequency radiation. Radio-frequencies (RF) of 39 MHz and other frequencies, IR, gas plasma radiation and ultra high frequency (microwave 2450 MHz) have been compared and found to be of equal effectiveness in reducing impermeable seeds of legumes (Nelson et al., 1964; Nelson and Wolf, 1964; Nelson et al., 1968; Stetson and Nelson, 1972) and in cotton (Stone et al., 1973). Treatments are more effective at low seed moistures and the effect is most rapidly accomplished by RF. Optimum radiation exposures for reduction of impermeable seed



percentages of legumes required elevation of seed temperatures to about 70-80°C and these temperatures were independent of initial seed temperature in the range -18 to 23°C (Ballard et al., 1976). There appeared to be no deleterious effects and the technique is inexpensive.

### B. Natural Softening

In nature, the seed coat may be broken down or punctured by mechanical abrasion, especially during cultivation, passage through the digestive tract of animals and birds, and by fire. Microbial attack has been suggested as a mechanism of softening (Raleigh, 1930; Pfeiffer, 1934; Trumble, 1937; and Mayer and Poljakoff-Mayber, 1975), although no quantitative data on the degree of softening has been presented. Raleigh (1930), on the basis of circumstantial evidence, suggested that the fungi Rhizopus may corrode the seed coat of Gymnocladus dioica by pectinase activity, while Brant et al. (1971) showed a small response in seed softening following hemicellulase or pectinase incubation of crown vetch.

High temperatures and temperature fluctuations are a major factor in softening seeds of winter annuals in Australia (Quinlivan, 1971a) and California (Williams and Elliott, 1960). In subterranean clover, Ornithopus compressus, Lupinus varius, and Stylosanthes humilies, natural softening under temperature fluctuations does not appear to commence until the moisture content of the seeds is low (Barrett-Lennard and Gladstones, 1964; Quinlivan, 1971a), e.g., less than 8.5% in Lupinus varius (Quinlivan, 1968). Neither the number of cycles of temperature fluctuations, nor the amplitude of the fluctuation (provided it was greater than 15°C) were important as compared to the maximum daily temperature to which these seeds

were exposed (Quinlivan, 1966; Hagon, 1971). In two species of vetch (Vicia sativa and V. grandiflora) with a high impermeable seed content, alternating temperatures between 4.5 and 21°C but not 21 and 32°C, resulted in the germination of 30 to 40% of the impermeable viable seeds. In V. angustifolia, both temperature regimes resulted in the germination of a high percentage of the impermeable seeds (Elkins et al., 1966).

Natural reversibility under high RH also occurs. Subterranean clover seeds with 10% moisture (dry weight basis) are conditionally hard and soften slowly in a humid atmosphere while seeds with less than 8.5% moisture are absolutely hard and will not soften in moist conditions, although temperature fluctuations are effective in softening. Thus, germination in the field of subterranean clover will depend on the degree of desiccation of the seed and the maximum temperature (Quinlivan, 1971a).

### Mechanism of Water Impermeability and Softening

The testa and its structures, the hilum, micropyle, strophiole, and chalaza, have all been implicated as barriers to water or as areas of weakness where imbibition occurs. The pathways of water movement into seeds have been traced most often by osmic acid (Hamly, 1932; Martin and Watt, 1944; Ballard et al., 1976), as well as by stains (Coe and Martin, 1920) and by iodine (Hyde, 1954).

Ontogeny studies indicate that water impermeability occurs late in the development of the seed (el Bagoury and Niyazi, 1973). It can be manipulated during seed ripening in Ononis sicula by changes in day-length (Gutterman and Heydecker, 1973) and by O<sub>2</sub> availability in wild pea, Pisum elatius (Marbach and Mayer, 1974).

A relationship between seed color and water impermeability

has been reported by Stewart (1926), Evenari et al. (1966), Gutterman and Heydecker (1973), and Marbach and Mayer (1974), although Gloyer (1932) found no such relationship in the pea and bean varieties he examined. In wild pea, both the browning of the seed coat and its impermeability depend on the presence of oxygen during the latter stages of seed desiccation. A similar trend was observed in the seeds of two other legumes, Cercis siligustrum and Robinia pseudacacia (Marbach and Mayer, 1974). The phenolic compounds of the seed coat may be involved in impermeability changes in the legume Hedysarum (Come and Semadeni, 1973). In peas, the results of Marbach and Mayer (1974, 1975) suggest that the impermeability of seed coats is associated with higher levels of phenolic compounds in the seed coat, and with their level of oxidation. The oxidation of the phenolics appears to be catalyzed by catechol oxidase which is very active in the seed coat of peas during desiccation and is  $O_2$ -dependent (Fig. 5). They considered that the browning or tanning reaction associated with impermeability is the result of quinone formation and the interaction of the quinones with each other and with proteins in

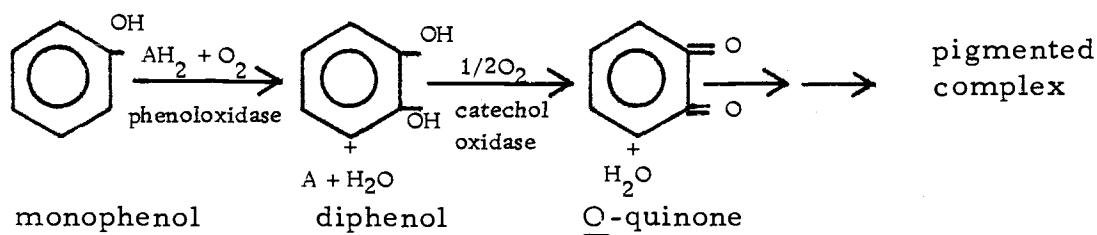


Fig. 5. Formation of waterproofing pigmented complex from monophenol during hard seed ontogeny.

the cells. The impermeable seeds of wild pea also had seed coats whose relative weights were three times greater than cultivated permeable P. sativum (Marbach and Mayer, 1974).

Correlations between seed color, cuticle development (determined by scanning electron micrographs), and water impermeability, have been observed (Gutterman and Heydecker, 1973; Marbach and Mayer, 1974).

There has been disagreement about which of the layers of the testa cause water impermeability. Pammel (1889) suggested that the waxy or fatty nature of the cuticle makes it repellent to water. The successful softening of seeds treated with ethyl alcohol and other polar substances has commonly been interpreted as the removal of the waxy layer of the testa (Mayer and Poljakoff-Mayber, 1975). However, in Gleditsia triacanthos (Veschaffelt, 1912) and field bindweed (Callihan, 1961), the softening of seed with alcohol was associated with disruption and water entry at the hilum. Thus, the waxy layer interpretation would not be valid unless the site of water entry was determined to be the whole seed testa. Further, in sweet clover (Coe and Martin, 1920), blue lupine (Burns, 1959), and soybean (Baciu-Miclaus, 1970) dyes penetrate the cuticle into the top of the palisade cells.

While Coe and Martin (1920) considered the light line to be the impermeable barrier, other authors considered the macrosclerid cells as the water impermeable barrier. Water permeability of Tetragonolobus conjugatus, T. requienii, and the pea seed coat may be influenced by specific suberin adcrustations which reinforce the walls situated on the outer ends of the epidermal palisade cells immediately under the cuticle (Spurny, 1963, 1972). The impermeability of alfalfa was attributed to the thick cell wall area in the outer end of the palisade tissue (Lute, 1928). In Trifolium, Medicago,

and Melilotus species, Spurny (1972) observed the characteristic thickening of the cell wall on the cell apex of the macrosclerids that result in the terminal caps of the palisade cells.

Raleigh (1930) considered that water impermeability in Gymnocladus dioica was a physical barrier, rather than a chemical barrier due to uniform shrinking of the testa until the macrosclerids are tightly oppressed. The action of sulfuric acid in softening seeds of Melilotus (Hamly, 1932) and crown vetch (Brant et al., 1971) appears to result from the localized removal of cuticle and dissolving of the macrosclerid caps. On the basis of scanning electron micrographs of liquid N<sub>2</sub> and boiling water treatments, it appears that differential thermal expansion separates the macrosclerid cells of the palisade layer, permitting water to penetrate.

Crown vetch seeds had to be punctured to a depth of 98 µm or more for rapid imbibition of water and subsequent germination. At this depth, penetration of the macrosclerid cells and other components of the outer integument had occurred, as well as the aleurone layer in addition to portions of the endosperm layer in some seeds (McKee et al., 1977). These findings support the suggestion by Ballard (1973) that relative impermeability persists well below the light line to deeper levels than was previously thought.

Various stains have been used to determine the chemical nature of the components of the macrosclerids and their caps. Pectin substances (Coe and Martin, 1920; Raleigh, 1930), lignin (Reeves and Valle, 1932; Winter, 1960; McKee et al., 1977) and suberin (Hamly, 1932; Aitken, 1939; Scott et al., 1962) have been reported in various species. In soybeans, Baciú-Miclaus (1970) considered the base of the macrosclerids to be the impermeable barrier because of the high level of lignification that occurred at the base of the cells and at the top of the columnar cells and the presence of large quantities of fat

present at the base of the macrosclerids and penetrating between cell walls. Extensive deposits of tannin-like materials occur just below the inner integument in crown vetch (McKee et al., 1977).

There have been virtually no attempts to quantify the various waterproofing components in the seed testa. Raleigh (1930) has reported that the macrosclerids of Gymnocladus dioica contained the equivalent of 22% calcium pectate on a dry weight basis.

The hilum is involved in the development of water permeability in Papilionoideae legumes (Hyde, 1954) and field bindweed (Callihan, 1961). The hilum fissure appears to act as a hygroscopic valve once the epidermis had become impermeable at about 25% moisture content. The hilum fissure, by virtue of the counter-palisade cells, opens when the atmospheric moisture is less than that of the seed, resulting in moisture loss from the seed. No loss of moisture occurred when Hyde (1954) covered the hilum with petroleum jelly. When the RH increased, the hilum fissure closed. However, the hilum fissure failed to respond to a gradual change in RH. While no mechanism of action has been proposed for the opening and closing of the hilum fissure in response to RH, it is possible that it is a physical process due to a differential rate of desiccation of the counter-palisade and palisade tissue of the hilum. In legume subfamilies other than the Papilionoideae, which generally lack counter palisade tissue, it is unclear whether the hilum functions in moisture loss or how moisture is lost once the testa becomes impermeable.

Certain softening treatments act on the hilum, allowing water to enter. Ethyl alcohol caused the counter-palisade layer in field bindweed to contract, opening the hilum aperture (Callihan, 1961). Sulfuric acid caused partial hydrolysis of the counter-palisade in Lupinus angustifolia (Burns, 1959) and was selective in dissolving the tissue around the hilum of field bindweed (Callihan, 1961). IR

treatment caused the counter-palisade layer to lift from the palisade layer in crimson clover (Te May Ching, pers. comm.), while heat irreversibly opened the hilum of varnish-leaf ceanothus (Gratkowski, 1962). Water entry in Lespedeza capitata (Hutton and Porter, 1937), and Melilotus alba (Martin and Watt, 1944) that had become permeable by percussion was via an opening, at the base of the hilum, where the vascular bundle had connected the seed to the parent plant.

The structural importance of the strophiole as an area of weakness where impermeable seeds of the Papilionoideae imbibe was demonstrated by Hamly (1932) and confirmed by Aitken (1939). More recently, studies in a number of species by Hagon and Ballard (1970), Hagon (1972), Ballard (1973), Ballard et al. (1976) have shown that percussion, temperature fluctuations, and RF of 39 and 2450 MHz all result in water imbibition via the strophiole.

The chalaza has been determined as the area of weakness in impermeable seeds of peas (Spurny, 1972), cotton (Simpson et al., 1940; Christiansen and Moore, 1959), and velvetleaf (LaCroix and Staniforth, 1964). Softening of cotton occurs if the chalazal plug is ruptured by hot water, ethyl alcohol, ether, or pierced by a small needle. Non-hard cotton seeds, characteristic of cultivated varieties, have a smaller, poorly organized chalazal cap which only occasionally adheres to the palisade layer and these seeds imbibe freely. Drying or heating of velvetleaf seeds results in a crescent-shaped rupture above the chalazal slit that renders the seed permeable to water (LaCroix and Staniforth, 1964).

The micropyle has been implicated in the moisture status of impermeable seeds of bean (Kyle, 1959), while fire-induced germination of Rhus ovata was associated with cracks along the seed coat above the micropyle (Stone and Juhren, 1951). In the softening of various desert Convolvulus species, percussion and acid treatments

loosen or dislodge the micropylar plug, according to Koller and Cohen (1959). They equate this with the structure described by Hamly (1932) but unfortunately, Koller and Cohen (1959) do not present illustrations of the structure they observed.

Scarification (Hamly, 1932) and RF treatments that heated various legume seeds above 120°C (Ballard et al., 1976) result in numerous cracks developing in the seed coat, allowing water to enter.

There is evidence that water impermeability is not an absolute condition, and can be reversed. Hagon and Ballard (1970) found that permeability in subterranean clover induced by percussion and resulting in the opening of the strophiole is not absolute, but can be reversed by subsequent storage at 20% RH. While Hamly (1932) was unable to demonstrate this reversibility during storage "for weeks in a dry place," Hagon and Ballard (1970) suggest that the RH may not have been low enough in relation to seed moisture content to permit drying. Nikolaeva (1969) also suggests that water entry may be reversible, as fluctuations in germination rates are observed in European yellow lupine and some species of clover in storage.

Reversibility of impermeability has been demonstrated in Lupinus varius (Gladstones, 1958; Quinlivan, 1968), Ornithopus compressus (Barrett-Lennard and Gladstones, 1964) and in a number of species from the genera Trifolium, Lupinus, Medicago, Astragalus, Lotus, and Melilotus (Nakamura, 1962). In all of the above examples, reversibility occurred at seed moisture content of about 10%. At lower seed moistures, impermeability is essentially irreversible during storage.



### Epilogue

On the basis of our present day information, a number of areas of research would extend our knowledge on water impermeable seed dormancy. (a) The comparative anatomy and biochemistry of the testa of hard seeds from various families, including structures involved in seed softening. (b) Detailed comparisons of lines within a species or variety selected for high and low percentage of impermeable seed. (c) Genetics of the impermeable characteristic, especially in agricultural crops that have hard seed. (d) The strophiole, its occurrence in different species and the use of this term in botanical literature needs to be clarified. (e) The ability to determine absolutely impermeable seeds that can be stored at high humidity without softening, from conditionally impermeable seeds which will soften at high humidities (Quinlivan, 1971a). The "depth" of impermeable seed dormancy appears to be a function of seed moisture and this should not be overlooked when treatments on impermeable seeds are being compared.

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III. Control of Volunteer Legumes During Establishment  
of Legume Seed Crops with Activated Carbon Bands  
and Non-Selective Herbicides. I. White Clover

ABSTRACT

In white clover (Trifolium repens L.) seed production, volunteer legumes arising from seed in the soil may result in contamination of seed lots. Experiments were conducted in western Oregon to determine the potential of using activated carbon applied in bands over the seed row, in conjunction with non-selective herbicides, as a method to control volunteers. The carbon:herbicide ratio required for protection from 0.5 kg/ha diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], atrazine [2-chloro-4-(ethylamino)-6-isopropylamino]-s-triazine], and simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] were 50:1, 200:1, and 200:1, respectively. The effective width of protection from a 25 mm-wide band of carbon was 13 mm and 18 mm using 4 kg/ha diuron with 150 and 300 kg/ha of carbon, respectively. In the field, when white clover was grown as a crop, seedling numbers were reduced to 64, 42, and 22% of the check by diuron at 2, 3, and 4 kg/ha, respectively. The checks averaged 100 seedlings/m<sup>2</sup>. This reduction resulted in seed yields of 80, 40, and 14% of the check at first harvest of fall-planted white clover. Atrazine and simazine at 1.25 and 1.5 kg/ha, respectively, reduced seedling counts to the same extent as 2 kg/ha of diuron. In the second harvest of these treatments, seed yields from plots established with 2 and 3 kg/ha of diuron were similar to the check. Spring-established seed yields were not reduced by diuron. White clover injury was greatest when diuron was applied to soil that was moist on the surface. Seed germination and seed weight were not influenced by the herbicides used in these experiments. Good weed control between the carbon bands was obtained



but grasses and broadleaf weeds were also protected by the carbon band. Using carbon banding, volunteer white clover and volunteer narrow birdsfoot trefoil (Lotus tenuis Waldst. & Kit.) were both reduced by 95% with diuron applied at 2.0 and 3.3 kg/ha diuron, respectively.

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Additional index words: Atrazine, Diuron, Simazine, Clover seed production, Row width, Trifolium repens.

## INTRODUCTION

In legume seed production, volunteer legumes arising from seed in the soil may result in contamination of seed lots. Two forms of varietal contamination are possible. Intervarietal contamination occurs between varieties of the same species (16, 18), while intravarietal contamination occurs between classes (breeders, foundation, registered, and certified) of the same variety. Varietal contamination may either be genetic (due to cross-pollination) or physical (due to the presence of the contaminating seed in the seed lot). Varietal contamination is difficult to identify, whether in the field or in seed samples. Species contamination occurs with other legume species that have similar sized seed.

Seed certifying agencies have established field history requirements to reduce the potential of varietal contamination by specifying that a certain number of years and cultivations must elapse between crops of different varieties of the legume. These limitations do not always prevent contamination because legumes produce water-impermeable (hard) seed, which may remain viable in the soil for 20 or more years (8). Further, high seed populations may develop in the soil.

One approach to reducing contamination from volunteer legumes in legume seed fields is the use of activated carbon bands and non-selective herbicides. Activated carbon readily adsorbs many different organic compounds (10, 19) and research papers on a wide range of uses of activated carbon in conjunction with herbicides have been listed (2). When activated carbon was sprayed in a slurry as a narrow band above the seed row, it protected perennial grasses (4, 14), subterranean clover (Trifolium subterraneum L.) (13) and rose clover (T. hirtum All.) (13) from preemergence non-selective herbicides. Varietal contaminants in Kentucky bluegrass (Poa pratensis L.) are also controlled (15). In 1977, an estimated 6,300 ha of perennial grass seed were established with activated carbon and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] in Oregon.

The percent control of volunteers and weeds in an activated carbon banding, non-selective herbicide system is often assumed to be equivalent to the percent area outside the carbon bands. However, a reduction in volunteers may occur within the band, while control between the bands may not be perfect. An estimate of the percent control of volunteers is:

$$V = 100 - A + 0.01 AB - 0.01 (100-C) (100-A) \quad [1]$$

$$\text{when } A = 100 E/R \quad [2]$$

where V is the percent control of volunteers, A is the percent band area, B is the percent reduction of volunteers within the band, C is the percent reduction of volunteers between bands, E is the band width, and R is the distance between crop rows. Herbicide rate, carbon rate, band width, white clover seeding rate, and row width will affect components of equations [1] and [2].

This paper deals with white clover (T. repens L.) which has seed similar in size to small hop clover (T. dubium Sibth.), strawberry clover (T. fragiferum L.), alsike clover (T. hybridum L.),

big trefoil (*Lotus pendunculatus* Cav.), and narrow birdsfoot trefoil (*L. tenuis* Waldst. & Kit.). Pastures where white clover had been harvested for seed within the previous 5 years averaged in the top 5 cm of soil 11,160 and 7,480 seeds/m<sup>2</sup> of white clover and small hop clover, respectively (11). Other studies report permanent grassland and arable fields with white clover seed populations ranging from 125 to 2,500 seeds/m<sup>2</sup> (5, 7, 11, 17).

The objectives of this study were (a) to determine the tolerance of white clover, established with activated carbon bands, to diuron and other herbicides, and (b) to estimate components of equations [1] and [2] in greenhouse and field experiments.

## MATERIALS AND METHODS

Greenhouse Experiments. Five experiments, labeled as Experiments 1 to 5, were conducted in 10 by 10 by 10-cm plastic pots. Temperatures were in the range of 24 to 28°C during the day and 16°C at night with a 13-hour daylength. A loam greenhouse soil mix, organic matter 3.8%, was steam-sterilized at least 2 weeks before planting. 'Grasslands Huia' white clover seeds were broadcast-sown (25 or 100 seeds) or sown in a single row (25 seeds) and were covered with 5 mm of soil. The pots were subsurface-irrigated until the moisture reached the soil surface and then allowed to drain for 6 to 12 hours before spraying. All subsequent applications of water were applied by subsurface irrigation.

All spray treatments were applied with a track-type sprayer. Activated carbon<sup>1</sup> was mixed with water, 60 g/liter, and sprayed

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<sup>1</sup> Gro-Safe Activated Carbon manufactured by ICI United States, Wilmington, DE 19897.

either broadcast or in a 2.5 cm band using a template. The herbicides diuron, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], simazine [2-chloro-4,6-bis(ethylamino)-s-triazine], and bromacil (5-bromo-3-sec-butyl-6-methyluracil) were applied pre-emergence in 225 liters/ha of water. Treatments were evaluated 6 weeks after planting by counting the number of surviving seedlings per pot and/or by determining the dry weight of the topgrowth. A randomized complete block design with three to five replicates was used in each experiment and each experiment was repeated.

In Experiment 1, the sensitivity of white clover to diuron, atrazine, simazine, and bromacil applied at 0, 0.5, 1.0, and 1.5 kg/ha was determined. Experiment 2 determined the rate of diuron and atrazine that reduced topgrowth dry weight by 50% ( $GR_{50}$ ) using a probit analysis technique (3, 9). In Experiment 3, white clover was established with 0, 150, and 300 kg/ha activated carbon in a 2.5-cm band and oversprayed with diuron at 0, 0.5, 1, 2, and 4 kg/ha. In Experiment 4, the carbon-to-herbicide ratio (w/w) for complete protection from diuron, atrazine, and simazine applied at 0.5 kg/ha was determined. In Experiment 5, the effective width of protection of a 2.5-cm band of activated carbon applied at 150 and 300 kg/ha and oversprayed with diuron applied at 0, 1, 2, and 4 kg/ha was determined.

Field Experiments. Eight field experiments, labeled as Experiments 6 to 13, were conducted at the Oregon State University Hyslop and Schmidt Farms on a Woodburn silt loam, a member of the fine-silty, mixed, mesic, Aquualtic Argixerolls, with 3.5 and 2.8% organic matter, respectively. Experiments were sown with rhizobia-inoculated 'Grasslands Huia' white clover at 4 kg/ha. Activated carbon<sup>1</sup> mixed with water at 60 g/liter was sprayed in a 3-cm wide band above the seed row at 400 kg/ha in the band, unless otherwise stated. A small tractor equipped with spray tank and four planters

was used for seeding and applying the carbon. The planters were mounted 30 cm apart with a drop nozzle mounted immediately behind the press wheel of each. Herbicides were applied within 24 hours of seeding in 225 liters/ha of water with a bicycle-wheel sprayer. Unless otherwise stated, diuron was applied at four rates, 0, 2, 3, and 4 kg/ha. The experimental design used in all experiments was a randomized block design with at least four replications. Plot size was 2.5 by 6.7 m.

Four to six weeks after seeding, 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] and pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide] were applied at 1.2 and 1.5 kg/ha, respectively, to control broadleaf and grass weeds that emerged within the carbon bands. Experiment 8 received pronamide only.

Seedling counts were made within 16 weeks of planting. Plots established for at least 12 months were clipped in the last week of April and dry matter yields were determined. Fall-seeded, but not spring-seeded, white clover produced seed the first summer. Honeybees (2 colonies/ha) were introduced after 20% bloom occurred. One irrigation of 50 mm water was applied during early bloom. Seed yields were determined by cutting a strip 0.9 by 6 m from the center of each plot. The plant samples were placed in large burlap bags, dried, double-threshed, and cleaned. Hyslop plots were harvested for seed in two consecutive years, 1977 and 1978. Schmidt plots were harvested in 1978 only.

Post-harvest management of plots included overspraying with 0.4 kg/ha paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) in late November and fertilizing with 30 kg/ha S, 2 kg/ha B, and 50 kg/ha P in February.

Seed germination and seed weight were determined for the first harvest at Hyslop of Experiments 7 and 8, and the 400 kg/ha carbon

treatment in Experiment 11. Four 100-seed lots per plot were weighed. The seeds were placed on moist filter paper in a dark germinator at 20°C. The germination percentage was determined at 7 days by counting 'normal' germinated plus hard seed.

Experiment 6 was established at Hyslop on 2 October 1977 as two identical trials, each with nine replications, to compare white clover growth and lateral spread of stolons from individual plants. Seed was sown in the center of 1 m<sup>2</sup> plots, protected by a 5 by 5 cm square of activated carbon and oversprayed with four rates of diuron.

Experiments 7 to 11 evaluated the tolerance of white clover to diuron and other herbicides in a carbon banding system. Experiment 7 was established at Hyslop on 11 May 1976, with 330 kg/ha activated carbon and 3 rates of diuron, 0, 2, and 4 kg/ha, with five replications. Experiment 8 was established at Hyslop on 20 September 1976 and 1 October 1977, with 400 kg/ha activated carbon. Four herbicides were applied, diuron at 2, 3, and 4 kg/ha, simazine at 1.5 and 2.5 kg/ha, atrazine at 0.75 and 1.25 kg/ha, and 2,4-DB at 1.2 kg/ha. In each block one row was seeded without carbon and seedling numbers in the within-carbon and without-carbon rows were compared. Experiment 9 was established at Hyslop on 20 September 1976 and at Schmidt on 1 April 1977. Four rates of diuron were applied to three adjacent blocks with different row spacings, 30, 40, and 50 cm, equivalent to seeding rates of 4, 3, and 2.4 kg/ha, respectively. Experiment 10 was established at Hyslop on 21 September 1976 and Schmidt on 2 April 1977, with four rates of diuron applied to three adjacent blocks with different seeding rates of white clover, 2, 4, and 22 kg/ha. Experiment 11 was established at Hyslop on 21 September 1976, and at Schmidt on 2 April 1977 with four rates of diuron applied to three adjacent blocks with different carbon rates, 200, 300, and 400 kg/ha.

Two experiments assessed the control of volunteer legumes in crops using activated carbon banding and diuron. Experiment 12 was established at Hyslop on 12 May 1976 and 2 October 1977 to determine the control of volunteer white clover in red clover established with 330 kg/ha activated carbon in 3 cm bands. White clover was broadcast-sown 2 days prior to the red clover at a rate of 1400 viable seeds/m<sup>2</sup> and incorporated to a depth of 7 cm. Three herbicides were applied, diuron at 2, 3, and 4 kg/ha, atrazine at 1, 1.5, and 2 kg/ha, and dichlobenil (2,6-dichlorobenzonitrile) at 4 and 6 kg/ha. Volunteer white clover seedlings were counted (1976) and percent control was visually estimated.

Experiment 13 assessed the control of Lotus tenuis volunteers at Hyslop in plantings of orchardgrass (Dactylis glomerata L. 'Potomac') or Kentucky bluegrass (Poa pratensis L. 'Newport') established with a chemical seedbed treatment alone or a combination chemical seedbed-carbon planting. The trial site was used for L. tenuis seed production from 1964 to 1970. The field was mechanically fallowed from 1971 to 1975 except for a period from October 1972 to August 1973, when the field was planted to winter wheat. Winter wheat was planted in October 1975 as a test species for evaluating several different chemical seedbed treatments. The chemical seedbed treatments (Table 6) included protham (isopropyl carbanilate), paraquat with 0.125% v/v non-ionic surfactant, and glyphosate [N-(phosphonomethyl)glycine]. The grasses were planted on 30 cm centers without further seedbed preparation on 12 March 1976. A split plot design with main plots being chemical seedbed or seedbed + carbon banding was used with six replications. Each subplot (herbicide treatments) was 2.4 by 5.5 m. The carbon-banded plots were established with 330 kg/ha activated carbon in 2.5 cm wide bands and were sprayed with 3.3 kg/ha diuron immediately after

planting.

## RESULTS AND DISCUSSION

Seedling Sensitivity to Herbicides. Without activated carbon protection, white clover seedlings were susceptible to low rates of herbicides applied preemergence. In the greenhouse, 0.5 kg/ha atrazine, simazine, or bromacil completely killed the seedlings. Diuron at 0.5 kg/ha reduced seedling numbers by 88 and 93% in two experiments, while 1 kg/ha killed all seedlings (Appendices 1 and 2). In one field experiment, the lowest herbicide rates tested (atrazine 0.75 kg/ha, simazine 1.5 kg/ha, and diuron 2 kg/ha) killed all seedlings (Appendix 3). In a second experiment, white clover volunteers between carbon bands were reduced from 119 per m<sup>2</sup> to 3, 0, and 0 per m<sup>2</sup> by 2 and 3 kg/ha diuron and 1 kg/ha atrazine, respectively (Table 1).

Carbon Protection. In the greenhouse, activated carbon applied at 150 and 300 kg/ha, protected white clover seedlings from diuron applied preemergence at rates up to 2 and 4 kg/ha, respectively (Appendix 2). The ratio of activated carbon to herbicide that prevented a significant reduction in seedling numbers was 50:1 for diuron and 200:1 for atrazine and simazine (Appendix 4). These ratios are the same as those reported by Ahrens (1) for oats protected with an unnamed brand of activated carbon. This fourfold difference was due, in part, to differences in the sensitivity of white clover to the two herbicides. The GR<sub>50</sub> for atrazine and diuron applied preemergence in the absence of carbon was 0.025 kg/ha and 0.06 kg/ha, respectively (Appendix 5). Further, Jordan and Smith (12) found no difference in the percent adsorption of atrazine and diuron by 21 different carbon products.



TABLE 1. Effect of three preemergence herbicides on the control of white clover volunteers.<sup>a</sup>

Herbicide	Rate	Seedling counts, 1976 <sup>b</sup>		Control of Volunteers <sup>c</sup>	
		Within bands	Between bands	1976	1977
	kg/ha	No. /m	No. /m <sup>2</sup>	— % —	
Check	0.0	4	119	0	0
diuron	2.0	0	3	98	95
	3.0	2	0	95	85
	4.0	0	0	100	99
atrazine	1.0	3	0	92	89
	1.5	1	0	98	97
	3.0	0	0	100	100
dichlobenil	4.0	1	32	71	33
	6.0	1	15	83	80
L. S. D. (0.05)		3	16	11	18

<sup>a</sup> Experiment 12.

<sup>b</sup> Counts made 30 days after sowing

<sup>c</sup> Visual evaluation 15 (1976) weeks and 25 (1977) weeks after sowing

Increased rates of diuron resulted in an effective carbon band that was narrower than the applied band of carbon (Table 2). Thus, volunteer legumes which germinate at the edge of the carbon band will not be protected from herbicide injury. If the effective band becomes too narrow because of low carbon rates, high herbicide rates, or a narrow applied band, crop injury may result, especially if the applied band is not precisely positioned above the seed row.

TABLE 2. Effective band width for white clover established with an activated carbon band and diuron.<sup>a</sup>

Carbon rate kg/ha	Diuron, kg/ha			
	0	2	3	4
	mm			
150	23.5	20.4	16.1	12.8
300	23.1	20.8	17.7	18.8

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L. S. D. (0.05) = 3.2 mm

<sup>a</sup> Experiment 5.

In the field, when single spaced plants spread beyond the area of carbon protection onto soil treated with 4 kg/ha diuron, a reduction in stolon length and fresh weight occurred (Fig. 1). At 2 and 3 kg/ha diuron, no reduction occurred. A trend towards a reduction in flower heads per plant was also observed at the 4 kg/ha rate (Fig. 1).

When white clover was established with carbon bands, there was a marked reduction in the seedling population with increasing rates of diuron (Fig. 2, Appendix 6). Atrazine and simazine at 1.25 and 1.5 kg/ha, respectively, reduced seedling counts to the same extent as 2 kg/ha of diuron (Appendix 3).

In experiments established in the fall of 1977, white clover was severely injured, particularly in those plots established when the soil surface was wet at the time of spraying (Table 3). A possible explanation for this is that water molecules compete with diuron for adsorption sites on organic matter and clay colloids. Under conditions when the soil surface is high in moisture, more diuron would be available in the soil solution to interact with the emerging seedlings.

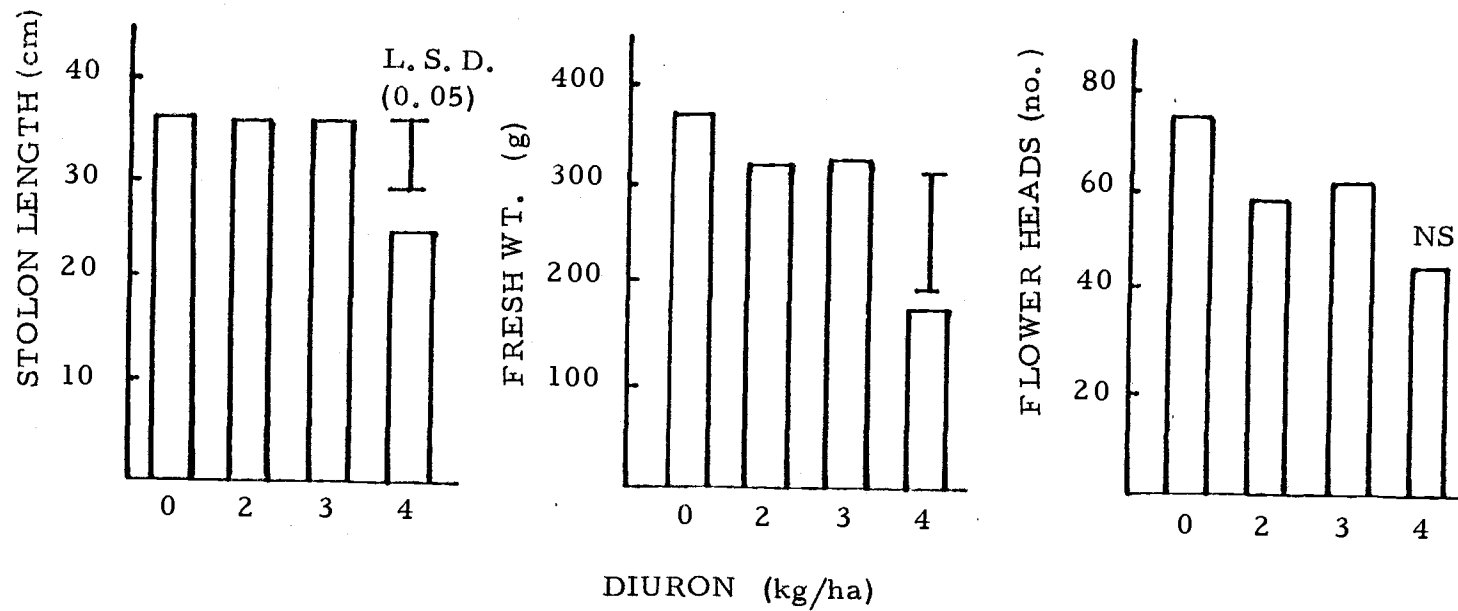


Fig. 1. Stolon length, fresh weight, and number of flower heads for single-spaced plants 40 weeks after establishment with activated carbon and diuron. Experiment 6.

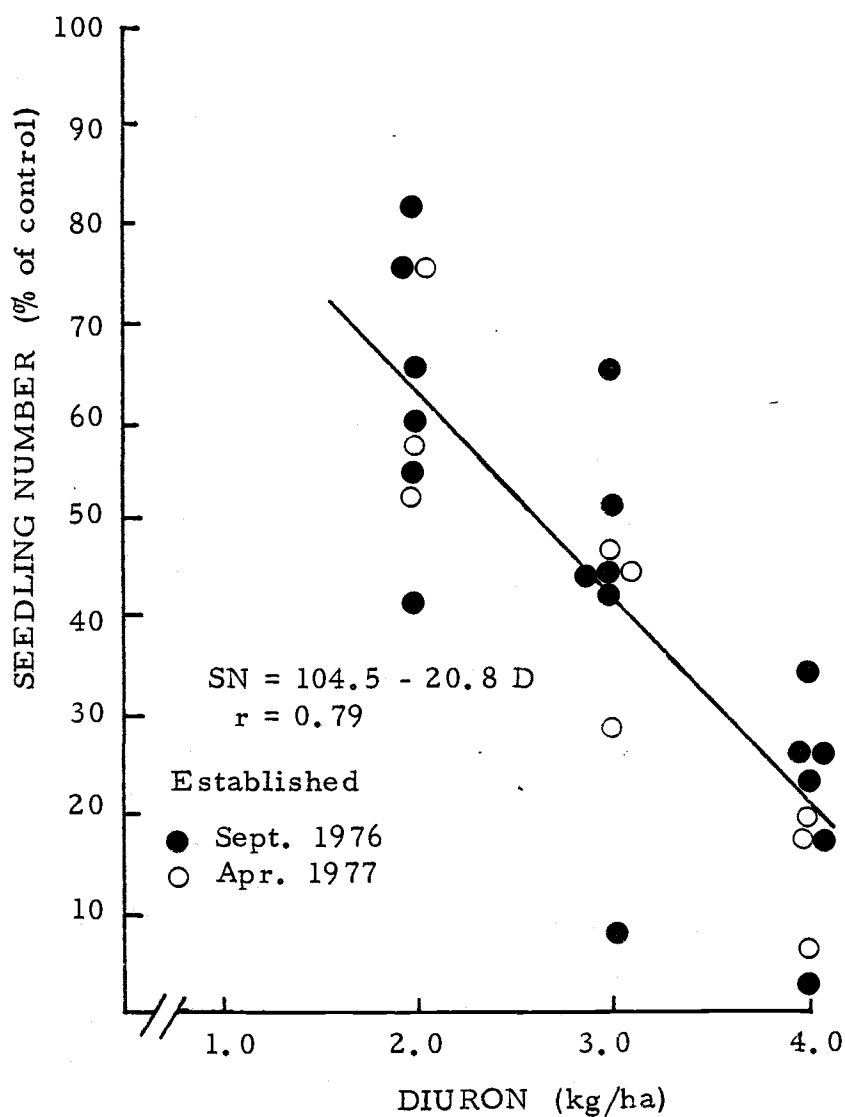


Fig. 2. The effect of diuron on seedling numbers (SN) of white clover established with carbon bands and three rates of diuron (D). Control plots established in September 1976 and April 1977 averaged 33 and 28 seedlings per meter of row, respectively. Row width 30 cm. Combined data from Experiments 8, 9, 10, and 11.

TABLE 3. Summary of environmental conditions associated with experiments established in four seasons.

	Spring 1976	Fall 1976	Spring 1977	Fall 1977	
Spraying date	13 May	20 Sept.	1 Apr.	2 Oct.	5 Oct.
Crop injury (%) <sup>a</sup>	20	25	20	95	58
Soil moisture at spraying	Dry	Dry	Dry on surface	Wet	Dry on surface
Precipitation (mm)					
before spraying					
4 weeks	47	48	138	198	196
after spraying					
1 week	0	0	1	17	17
4 weeks	82	30	26	61	72
Frost - 2 C (days from planting)	197	67	222	48	45
Soil temperature (C) <sup>b</sup>	16.6	17.5	12.7	13.1	13.1

<sup>a</sup> Visual evaluation of white clover established with 300-400 kg/ha activated carbon and 2 kg/ha diuron.

<sup>b</sup> Average daily soil temperature at 5 cm, during 4 weeks after spraying.

Also, diuron-injured seedlings may be more sensitive to frost heaving. Injury was greater in fall 1977 when higher soil moisture levels were associated with fewer days between planting and the first frost of -2°C (Table 3). Burr et al. (4), in a greenhouse study, reported no effect of soil moisture from 0 to 75% of field capacity on the ability of carbon to inactivate diuron. However, the effects of soil moisture

levels higher than 75% of field capacity and surface soil moisture were not included in their study. Chandler et al. (6) reported that cotton established with carbon bands and diuron was more severely injured in a year when the 5 cm soil temperatures during emergence were 4 C higher than in the year with lower soil temperatures. In our experiments, higher soil temperatures did not appear to be associated with increased injury (Table 3).

Seed Yields. The first harvest seed yields of white clover established in fall 1976 were reduced at all rates of diuron except the 22 kg/ha seeding rate plots (Table 4). There was a trend towards higher first harvest yields with narrower row widths, higher seeding rates, and higher carbon rates in the presence of diuron. In contrast, the yields of spring-established white clover were not reduced by diuron (Table 4, Appendix 7) although there was a trend for 4 kg/ha diuron to reduce yields. Both stands had a similar level of seedling reduction 14 weeks after seeding (Fig. 2). The difference in yield between the spring- and fall-established trials may reflect differences in stand age. At first harvest, the fall 1976 trial had been established for 10 months, compared with 16 months for the spring 1977 trial.

Recovery from Injury. A comparison of percent ground cover prior to harvest and seed yields at 10 and 22 months from establishment of the fall 1976 trials demonstrated the ability of white clover, with its stoloniferous growth habit, to recover from diuron injury (Fig. 3). At the 22-month harvest of this trial (Appendix 8), we could predict from regression analysis that seed yields would equal control yields, even when seedling numbers had been reduced during establishment to 23% of the control (Fig. 4). We could predict from regression analysis of the spring 1977 trial that seed yields would equal or exceed the control yields, even when seedling numbers had been reduced to 40% of the control (Appendix 9).

TABLE 4. First harvest seed yields of white clover established at different row widths, seeding rates and activated carbon rates.

Trial established	Diuron rate	Row width, cm			Seeding rate, kg/ha			Carbon rate, kg/ha		
		30	40	50	2	4	22	200	300	400
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		<hr/>								
		kg/ha								
		<hr/>								
Sept. 1976 <sup>a</sup>	0	582	485	443	503	461	452	594	476	461
	2	482	340	357	374	371	471	251	376	371
	3	234	79	97	254	129	421	9	248	129
	4	118	53	29	138	56	132	0	39	56
L. S. D. (0.05)		92	106	105	173	85	93	216	76	76
Apr. 1977 <sup>b</sup>	0	447	425	471	447	540	463	490	386	452
	2	471	378	494	461	461	493	515	388	483
	3	445	428	428	430	437	463	483	423	447
	4	460	380	445	391	474	452	430	351	439
L. S. D. (0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS

<sup>a</sup> Established September 1976, harvested July 1977. Experiments 9, 10, and 11, respectively.

<sup>b</sup> Established April 1977, harvested July 1978.

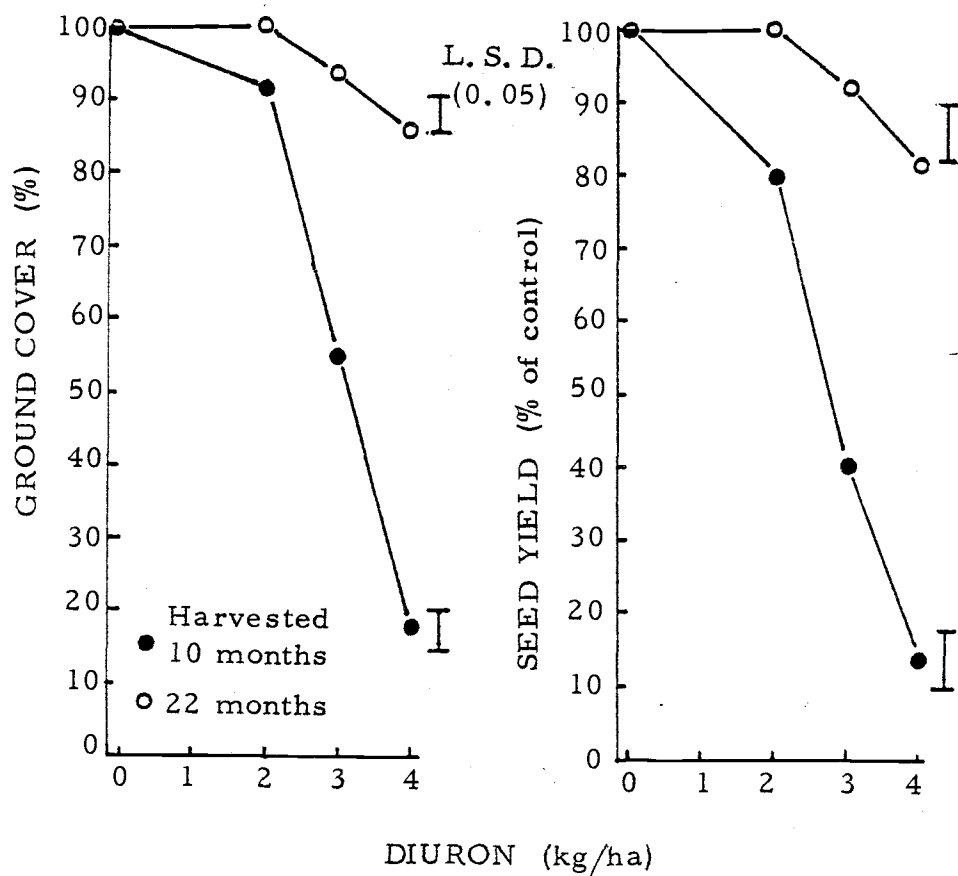


Fig. 3. Recovery of ground cover and seed yield from diuron injury in white clover established in September 1976 with activated carbon. Combined data from Experiments 8, 9, 10 and 11.



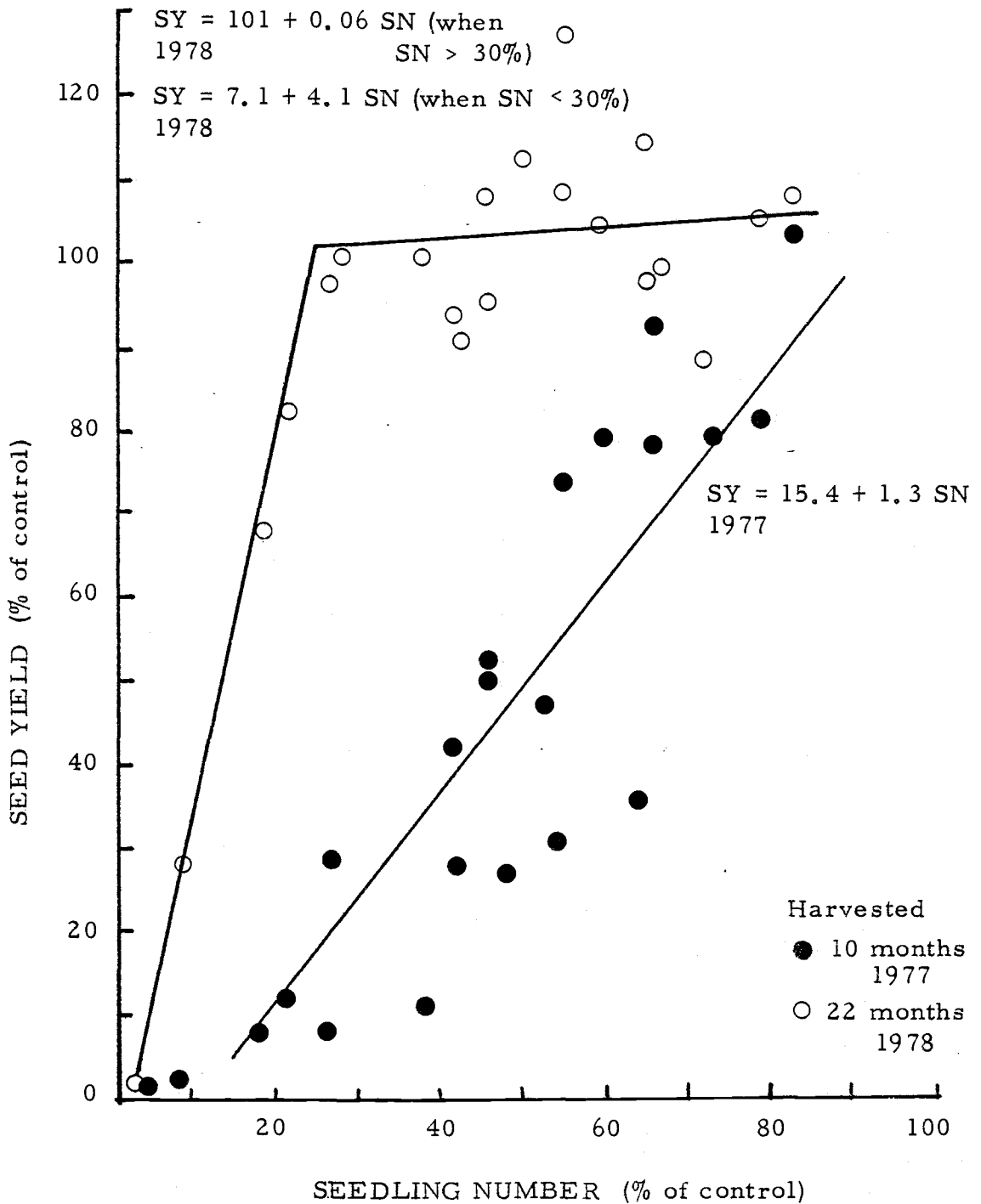


Fig. 4. Relationship between seedling numbers (SN), 15 weeks after seeding in September 1976 and seed yields (SY) of the first and second harvest. Seedling numbers in the check, 33 per meter of row. Row width, 30 cm. Combined data from Experiments 8, 9, 10 and 11.

Forage yields from plots cut in early spring also demonstrate the ability of white clover to recover from early stand reductions caused by herbicides. Forage yields from 87 treatments of spring- and fall-established white clover 14 to 20 months after establishment averaged ( $\pm$  SE)  $1933 \pm 708$  kg/ha. We could predict from regression analysis that forage yields would equal or exceed the control yields, even when the herbicide treatments reduced seedling numbers to 42% of the control (Appendix 9). Significant reductions in forage yields occurred with 2.5 kg/ha simazine, with 4 kg/ha diuron in some experiments, and at the low carbon rate (200 kg/ha) in the fall with 3 and 4 kg/ha diuron (Appendix 10).

Seed Quality. There were no differences in the germination percent or the seed weight of white clover harvested from plots established with atrazine, simazine, or diuron at rates up to 1.25, 2.5, and 4 kg/ha, respectively. The germination percent averaged ( $\pm$  SE)  $95 \pm 1\%$  (Appendix 11), and the seed weight averaged ( $\pm$  SE)  $663 \pm 15$  mg per 1000 seeds (Appendix 12).

Weed Control. In the fall, preemergence herbicides gave excellent control of all weed species germinating between the rows. In the spring trial, a different set of weeds was present and control of these was moderate, with good control of mayweed (Anthemis cotula L.) (Table 5), which is a serious problem in white clover seed production. In Experiment 8, the germination of weeds in the carbon band of plots treated with preemergence herbicides but not oversprayed with 2,4-DB, was sufficient to cause severe harvesting problems. Diuron and other preemergence herbicides used in carbon banding must be supplemented by herbicides that will control grass and broadleaf weeds that germinate in the carbon band.

Control of Volunteer Legumes. Carbon banding with diuron or atrazine controlled 85 to 100% of white clover volunteers (Table 1).

TABLE 5. Weed control in fall- and spring-sown white clover established with carbon bands and diuron.

Diuron kg/ha	Weed control between rows						Total weed control		
	Sept. 1976 <sup>a</sup>						Apr. 1977 <sup>a</sup>		
	PA <sup>b</sup>	MM	SV	LA	CB	RS	LM	AC	RS
0	0	0	0	0	0	0	0	0	0
2	88	99	79	89	99	97	39	89	62
3	93	100	85	98	100	100	55	96	82
4	99	100	96	99	100	100	73	98	92
L. S. D. (0.05)	5	1	10	7	1	4	24	8	11

<sup>a</sup> Visual evaluation made 16 and 7 weeks, respectively, after sowing.

<sup>b</sup> PA = Poa annua; MM = Matricaria matricarioides; SV = Senecio vulgaris; LA = Lamium amplexicaule; CB = Capsella bursa-pastoris; RS = Raphanus sativus; LM = Lolium multiflorum; AC = Anthemis cotula.

Dichlobenil gave inadequate control of volunteers. In the narrow birdsfoot trefoil trial, counts of volunteer plants ranged from 1 to 9 plants/m<sup>2</sup> in the chemical seedbed treatment (Table 6). When carbon banding with diuron was superimposed on the chemical seedbed, complete to nearly complete control of volunteers was achieved.

Using equations [ 1 ] and [ 2 ], we estimate that control of volunteer legumes in white clover will be 95% or greater when the row width is 50 cm, the carbon band width 2.5 cm, the percent reduction of volunteers within the band is 75%, and the percent reduction of volunteers between bands is 96%. This would require 20 kg/ha of activated

TABLE 6. Volunteer narrow birdsfoot trefoil counts in a chemical seedbed alone and with activated carbon plus diuron.<sup>a</sup>

Herbicide	Rate	Application date	Chemical seedbed	Chemical seedbed + carbon seeding
	kg/ha		plants/m <sup>2</sup>	
propham +	4.4 +	Nov. 1975		
paraquat	0.28	Mar. 1976	1.1	0
paraquat +	0.38 +	Nov. 1975		
paraquat	0.28	Mar. 1976	1.0	0
glyphosate	0.28	Feb. 1976	9.0	0.12
glyphosate	0.41	Feb. 1976	7.4	0.02
glyphosate	0.55	Feb. 1976	7.0	0
glyphosate	0.83	Feb. 1976	5.2	0
glyphosate	1.11	Feb. 1976	3.7	0
L. S. D. (0.05)			2.9	

<sup>a</sup> Counts made Nov. 1976. Experiment 13.

carbon applied at 400 kg/ha in a 2.5 cm band with 2 kg/ha diuron. Control of volunteers within the carbon band will increase beyond the initial herbicide effect because of competition between the crop and contaminant. Further, if a spot application of carbon is used (6) rather than the continuous carbon band used in this study, an additional increase in the percent control of volunteers would be expected.

## CONCLUSIONS

Under the conditions of these experiments, white clover can be successfully established using activated carbon bands with diuron at rates of 2 kg/ha, except when soils are wet on the surface. Diuron reduces seedling numbers at establishment. This reduction is often associated with a reduction in white clover seed yields at the first harvest. At the second harvest, a reduction in seed yield is not expected when the initial seedling counts exceed 25 seedlings/m<sup>2</sup> during establishment. Control of 95 to 99% of volunteer white clover can be expected using carbon bands in conjunction with diuron at 2 kg/ha.

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IV. Control of Volunteer Legumes During Establishment of  
Legume Seed Crops with Activated Carbon Bands and  
Non-Selective Herbicides. II. Red Clover and Alfalfa

ABSTRACT

In red clover (Trifolium pratense L.) and alfalfa (Medicago sativa L.) seed production, volunteer legumes may result in contamination of seed lots. Experiments were conducted in western Oregon to determine the potential of using activated carbon applied in bands over the seed row, in conjunction with non-selective herbicides to control volunteer legumes. Greenhouse and field experiments demonstrated that both red clover and alfalfa can be established with carbon bands and diuron [3-(3,4-dichlorophenyl)1,1-dimethyl urea]. Red clover tolerance to diuron varied with the season of establishment, with greatest injury occurring when diuron was applied in the fall to soils that were wet on the surface. Seed yields for red clover established with carbon bands and 0, 2, and 4 kg/ha diuron averaged, across all treatments, 566, 560, and 348 kg/ha, respectively. Seed yields for the two legumes established at 30-, 50-, and 80-cm row widths were: alfalfa, 264, 354, and 424 kg/ha, respectively; and red clover 635, 584, and 376 kg/ha, respectively. In the greenhouse, EPTC (S-ethyl dipropylthiocarbamate) granules at 10 mg/m of row applied as a mixture with the alfalfa seed, controlled perennial ryegrass (Lolium perenne L.) in the carbon band without reducing the number or weight of the alfalfa seedlings. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] or diuron at 1 and 3 kg/ha, respectively, controlled 85 to 99% of red clover volunteers.

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Additional index words: Atrazine, Diuron, EPTC, Simazine, Clover seed production, Row width, Medicago sativa, Trifolium pratense.



## INTRODUCTION

Volunteer legumes arising from seed in the soil may result in contamination of red clover (Trifolium pratense L.) and alfalfa (Medicago sativa L.) seed lots. In an earlier paper (7), we discussed three types of contamination and reported on investigations using carbon banding techniques and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] to reduce such contaminations in white clover. Inter-specific contamination occurs between species with similar seed size, including red clover, alfalfa, birdsfoot trefoil (Lotus corniculatus L.), white sweet clover (Melilotus alba Desr.), yellow sweet-clover (M. officinalis (L.) Lam.), and strawberry clover (Trifolium fragiferum L.).

The objectives of this study were (a) to determine the tolerance of both red clover and alfalfa, established with activated carbon bands, to diuron and other herbicides, and (b) to determine the potential of this technique for controlling volunteer legume contaminants. Alfalfa had previously been successfully established by this technique (5) using a methylthio-s-triazine herbicide. However, atrazine [2-chloro-4-(ethylamino)-6-isopropylamino)-s-triazine] had been found to be too injurious, although the rate of activated carbon was only 100 kg/ha in the band.

## MATERIALS AND METHODS

Greenhouse Experiments. Four experiments labeled as Experiments 1 to 4, were conducted using the same general methods outlined previously (7). 'Kenland' red clover, 'DuPuits' alfalfa, 'Manhattan' perennial ryegrass (Lolium perenne L.), and 'Grasslands Huia' white clover (T. repens L.) were sown and covered with 5 mm of

soil. Treatments were evaluated 5 to 6 weeks after planting by counting the number of surviving seedlings per pot and/or determining the dry weight of the topgrowth. A randomized complete block design with three to five replicates was used in each experiment. Experiments 1, 2, and 4 were repeated.

In Experiment 1, the rate of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] that reduced the topgrowth dryweight by 50% ( $GR_{50}$ ) of white clover, red clover, alfalfa, and perennial ryegrass was determined by using probit analysis (1, 4). Experiment 2 compared the root and shoot growth of alfalfa, white clover, and red clover. Four seeds of each species were planted at a depth of 5 cm adjacent to the glass surface of 30-cm deep, root observation boxes. The glass was protected with a light-proof, removable cover and the boxes were slanted at 30° from the vertical during the experiment. Two seedlings of each species were observed during a 21-day period. Root growth was traced onto clear plastic sheets. Fifty seeds of each species were also sown in pots and the number of days for 50% emergence was calculated. Experiment 3 compared seedling numbers of red clover and alfalfa established with 0, 150, and 300 kg/ha activated carbon mixed with water, 60 g/liter, and sprayed in a 3-cm band using a template. Diuron was applied preemergence at 0, 1, 2, 3, and 4 kg/ha. Experiment 4 evaluated the use of EPTC (S-ethyl dipropylthiocarbamate) to control perennial ryegrass volunteers within the carbon band. The treatments are listed in Table 7. Fifty alfalfa seeds were placed in a row and 100 perennial ryegrass seeds were broadcast in the pots. EPTC granules (10% active ingredient) were seeded with the alfalfa at 10 and 20 mg/m of row (equivalent to 2 and 4 kg EPTC/ha, respectively, assuming EPTC diffuses to form a band 5-cm wide). The seeds and EPTC were covered with 20 mm of soil.

Field Experiments. Five field experiments, labeled as Experiments 5 to 9, were conducted at the Oregon State University Hyslop and Schmidt Farms on a Woodburn silt loam, a member of the fine-silty, mixed, mesic, Aquualtic Argixerolls, with 3.5 and 2.8% organic matter, respectively. The same general methods of carbon seeding described previously were used (7). Experiments were sown with rhizobia-inoculated 'Kenland' red clover or 'DuPuits' alfalfa. Unless otherwise stated, diuron was applied at four rates, 0, 2, 3, and 4 kg/ha. All experiments were a randomized complete block design with at least four replications. Plot size was 2.5 by 6.5 m.

Four to six weeks after seeding, 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] and pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide] were applied at 1.2 and 1.5 kg/ha, respectively, to control broadleaf and grass weeds that emerged within the carbon bands. Experiment 6 received pronamide only.

Seedling counts were made within 16 weeks of planting. Plots were clipped in late May and dry matter yields were determined. Spring 1976, but not spring 1977, seeded red clover produced seed the first summer. A delay in the start of fall rains allowed the spring 1976-sown red clover to be harvested in October 1976, one month later than the normal harvest date. Alfalfa seed was harvested the first summer after fall plantings. Honeybees (2 colonies/ha) were introduced after 20% bloom occurred. Bumblebees (Bombus spp.) were also plentiful during the flowering period. The plots were irrigated during the bloom and early seed-filling period. Seed yields were determined from a strip 0.9 by 6 m cut from the center of each plot. Hyslop plots established in 1976 were harvested for seed in two consecutive years. Schmidt plots were harvested in 1978 only.

Post-harvest management of plots included overspraying with 0.5 kg/ha paraquat (1,1'-dimethyl-4,4'-bipyridium ion) in late

November and 1.75 kg/ha diuron in mid-December and fertilizing with 30 kg/ha S, 2 kg/ha B, and 50 kg/ha P in February.

Seed germination and seed weight of red clover were determined for the first harvest at Hyslop of Experiments 5 and 6 and the 300 kg/ha carbon treatment in Experiment 8.

Experiments 5 to 8 evaluated the tolerance of red clover and alfalfa to diuron and other herbicides in a carbon banding system. Experiment 5 was established with red clover at Hyslop on 11 May 1976 with 330 kg/ha activated carbon and 3 rates of diuron, 0, 2, and 4 kg/ha in a randomized block design with five replications. Experiment 6 consisted of two separate but adjacent trials with red clover and alfalfa established at Hyslop on 20 September 1976 with 400 kg/ha activated carbon. Four herbicides were applied, diuron at 2, 3, and 4 kg/ha, simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] at 1.5 and 2.5 kg/ha, atrazine [2-chloro-4-(ethylamino)-6-isopropylamino-s-triazine] at 0.75 and 1.25 kg/ha, and 2,4-DB at 1.2 kg/ha. In each block, one row was seeded without carbon and seedling numbers in the within-carbon and without-carbon rows were compared. Experiment 7 consisted of two separate but adjacent trials of red clover and alfalfa established at Hyslop on 20 September 1976 and at Schmidt on 2 April 1977. Four rates of diuron were applied to three adjacent blocks with different row widths, 30, 50, and 80 cm, equivalent to seeding rates of 4, 2.4, and 1.5 kg/ha, respectively. Experiment 8 was established with red clover at Hyslop on 23 September 1976 and 3 October 1977, and at Schmidt on 3 April 1977, with four rates of diuron applied to three adjacent blocks with activated carbon rates of 200, 300, and 400 kg/ha.

Experiment 9 was established at Hyslop on 12 May 1976 and 4 October 1977 to determine the control of volunteer red clover in white clover established with 330 kg/ha activated carbon in 3-cm

bands. Three herbicides were applied, diuron at 2, 3, and 4 kg/ha, atrazine at 1, 1.5, and 2 kg/ha, and dichlobenil (2,5-dichlorobenzo-nitrile) at 2, 4, and 6 kg/ha. The red clover was broadcast-sown 2 days prior to the white clover at a rate of 500 viable seeds/m<sup>2</sup> and incorporated to a depth of 7 cm. In 1976, volunteer red clover seedlings were counted between the rows and within the rows 10 weeks after seeding.

## RESULTS AND DISCUSSION

Species Sensitivity to Diuron. The GR<sub>50</sub> values for preemergence diuron applications in the greenhouse were alfalfa 0.59 kg/ha, perennial ryegrass 0.29 kg/ha, red clover 0.09 kg/ha, and white clover 0.06 kg/ha (Appendix 5). Among the three legumes, increased tolerance to diuron is correlated with larger seed size and greater vigor as indicated by rapid emergence and greater initial growth (Table 1). In the greenhouse, 2 kg/ha diuron reduced seedling numbers of red clover and alfalfa by 97 and 95%, respectively. In one field trial, the lowest diuron rate tested (2 kg/ha) killed all red clover seedlings, but not all alfalfa seedlings (Table 2).

Carbon Protection of Red Clover. Preliminary greenhouse data showed that there were no differences in seedling counts of red clover protected by 150 or 300 kg/ha of activated carbon from diuron applied at rates up to 4 kg/ha (Appendix 13). In the field, diuron at 2, 3, and 4 kg/ha reduced the number of seedlings that established (Table 3). The reduction in seedling numbers was greatest in plots established in October 1977, and was associated with a wet soil surface at the time of herbicide application. This is discussed in detail in a previous paper (7).

Diuron at 2 kg/ha did not reduce forage yields (Table 4) or seed

TABLE 1. Growth parameters of three legume species.<sup>a</sup>

Species	1000 Seed weight	50% Emergence	Shoot <sup>b</sup>		Root <sup>b</sup>		
			Leaves	Dry wt.	Laterals	Dry wt.	Length
	mg	days	no.	mg	no.	mg	cm
White clover	660	7.8	1.31	2.3	1.5	2.5	7.1
Red clover	1950	5.8	1.94	7.5	9.1	4.4	14.0
Alfalfa	2209	3.2	2.75	12.5	10.4	5.3	20.7
L. S. D. (0.05)			0.31	2.0	2.6	2.0	2.1

<sup>a</sup> Experiment 2.

<sup>b</sup> Measured 21 days after seeding.

TABLE 2. Effect of three herbicides on red clover and alfalfa established with activated carbon bands.<sup>a</sup>

Herbicide	Rate kg/ha	Red clover				Alfalfa		
		Seedling counts <sup>b</sup>		Seed yield		Seedling counts <sup>b</sup>		Seed yield
		With carbon	Without carbon	1977	1978	With carbon	Without carbon	1977
		—Seedlings/m—		—kg/ha—		—Seedlings/m—		—kg/ha—
Check	0	21	23	587	575	34	33	111
diuron	2.0	18	0	453	530	27	6	136
	3.0	16	0	426	518	29	4	122
	4.0	15	0	338	538	29	2	102
simazine	1.5	18	0	318	550	33	1	122
	2.5	17	0	190	533	31	0	113
atrazine	0.75	17	0	342	577	27	1	113
	1.25	17	0	377	508	32	0	104
L. S. D. (0.05)		6		127	NS	5	3	NS

<sup>a</sup> Established 16 Sept. 1976, Experiment 6.

<sup>b</sup> Evaluated 11 Nov. 1976

TABLE 3. Seedling numbers of red clover established with carbon bands.<sup>a</sup>

Diuron	Established	Sept. 1976		Apr. 1977		Oct. 1977	
rate	Evaluated	Jan. 1976	Sept. 1977	June 1977	Sept. 1978	Nov. 1977	Sept. 1978
kg/ha					No. /m		
0		39	16	30	7	32	11
2		29	11	19	7	20	5
3		28	11	13	7	11	3
4		25	9	12	7	6	1
L. S. D. (0.05)		4	1	6	NS	3	1

<sup>a</sup> Data are averages of three carbon rates; row width = 30 cm, seeding rate 4 kg/ha, equivalent to 54 viable seeds per meter of row. Experiment 8.



TABLE 4. Dry weight forage yields of red clover established with carbon bands.

Diuron rate	Established	May 1976 <sup>a</sup>	Sept. 1976 <sup>a</sup>		Apr. 1978 <sup>a</sup>
	Harvested	May 1977	May 1977	May 1978	May 1978
kg/ha		kg/ha			
0		4589	1986	3750	3939
2		4557	1510	4189	4039
3		--	1106	3989	3980
4		2077	716	3782	4012
L. S. D. (0.05)		702	230	NS	NS

<sup>a</sup> Means of 1, 6, and 6 trials, respectively.

yields (Table 5) of red clover, except forage yields of plots established in September 1976. This reduction in forage was not due to a reduction in red clover, but to a higher proportion of weeds in the untreated compared to the diuron-treated plots (Appendix 14). The effect of higher rates of diuron on forage and seed yields was variable for different times of establishment. There was no reduction in seed yields from plots established in April 1977, while 4 kg/ha diuron caused a severe reduction in seed yields from plots established in October 1977 (Table 5). This reduction was associated with greater reductions in seedling numbers because of diuron treatments (Table 3).

Second Seed Crop. Red clover in western Oregon normally has a stand life of 2 years, with the second crop producing lower yields than the first crop. At the second harvest, plots established with 2 and 3 kg/ha diuron showed a trend towards lower yields compared to

TABLE 5. First and second seed crop yields of red clover established with carbon bands and diuron.

Diuron rate	Established	May 1976 <sup>a</sup>		Sept. 1976 <sup>a</sup>		Apr. 1977 <sup>a</sup>	Oct. 1977 <sup>a</sup>
	Evaluated	Oct. 1976	Aug. 1977	Aug. 1977	Aug. 1978	Aug. 1978	Aug. 1978
kg/ha		kg/ha					
0		487	424	588	547	506	844
2		575	387	576	509	545	768
3		-	-	581	508	564	389
4		226	231	484	487	561	103
L. S. D. (0.05)		106	82	44	38	NS	99

<sup>a</sup> Means of 1, 7, 6, and 3 trials, respectively.

the check (Table 5). However, in one trial, the yield of the seed crop was higher than the first seed crop (Table 2).

Row Widths and Carbon Rates. Red clover was established at three row widths. As row width is increased, better volunteer control would be expected because the proportion of the field covered with carbon is decreased. In these experiments, the ratio of forage yields from 30-, 50-, and 80-cm row widths were 100, 98, and 66. Seed yields from 80-cm rows were lower than seed yields from 50- or 30-cm rows (Fig. 1, Appendix 15). Clifford (2) reported higher seed yields from 60-cm compared to 30-cm rows.

There appears to be no difference in seed yields when averaged across diuron rates from plots established with rates of 200, 300, or 400 kg/ha activated carbon in the band (Fig. 1, Appendix 15).

Carbon Protection of Alfalfa. Preliminary greenhouse data showed there were no differences in the number of surviving seedlings of alfalfa protected by 150 or 300 kg/ha of activated carbon from pre-emergence diuron applications at rates up to 4 kg/ha (Appendix 16). In the field there were only slight reductions in the number of alfalfa seedlings in plots treated with diuron, atrazine, and simazine (Table 2). Seed and forage yields of alfalfa established with carbon bands were not reduced by diuron (Table 2, 6). Of the three row widths tested, 80-cm widths gave lower forage yields but higher seed yields than the narrower widths tested (Table 6). This seed yield response to wider rows is consistent with other research (6). At Hyslop late-germinating weeds not controlled by 2,4-DB caused a reduction in plant height (Table 6).

Seed Quality. There were no differences in the germination percent ( $93 \pm 2\%$  SE) of red clover established with atrazine, simazine, or diuron at rates up to 1.25, 2.5, and 4.0 kg/ha, respectively (Appendix 17). The thousand seed weight for all treatments was

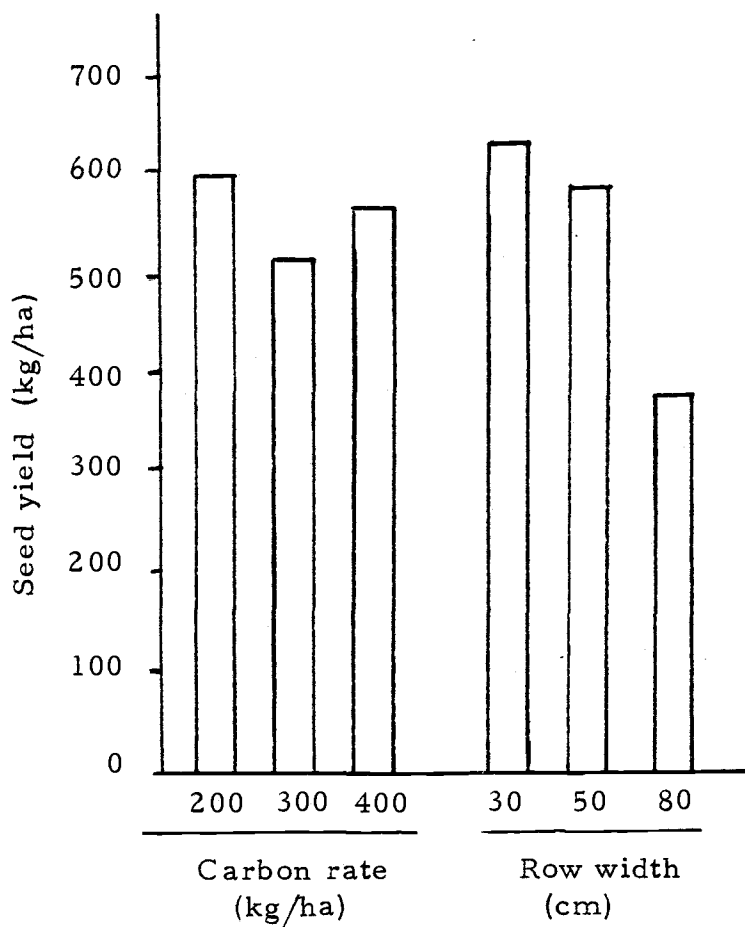


Fig. 1. Effect of row width and carbon rate on first harvest red clover seed yields. Carbon rates are means from three seasons and four diuron rates. Row widths are means from two seasons and four diuron rates.

TABLE 6. Alfalfa seed yield, forage dry matter, percent weeds, and height of alfalfa, established with carbon bands.<sup>a</sup>

Row width	Diuron	Hyslop					Schmidt
		Seed <sup>a</sup>	Forage		Height <sup>c</sup>	Weeds <sup>d</sup>	Forage
			1st cut <sup>b</sup>	2nd cut <sup>c</sup>			1st cut
cm		kg/ha			cm	%	kg/ha
30	0	282	1,539	4,486	31	33	5,914
	2	260	1,973	3,700	44	7	4,485
	3	227	1,640	3,800	41	3	5,015
	4	288	1,919	4,161	42	5	6,122
	L. S. D. (0.05)	NS	NS	NS	5	4	NS
	Mean	264	1,767	4,047			5,384
50	0	341	1,142	4,013	33	40	3,768
	2	321	1,580	4,298	43	9	4,882
	3	321	1,445	4,207	45	3	4,405
	4	433	1,268	4,135	43	7	4,735
	L. S. D. (0.05)	76	205	NS	5	4	NS
	Mean	354	1,359	4,163			4,448
80	0	348	1,454	3,781	34	42	4,132
	2	458	1,346	4,179	48	8	3,748
	3	460	1,160	3,815	47	5	3,149
	4	432	1,220	3,996	47	4	4,046
	L. S. D. (0.05)	NS	NS	NS	4	5	NS
	Mean	425	1,269	3,943			3,775

<sup>a</sup> Harvest 14 Sep. 1977. Experiment 7.

<sup>b</sup> Harvest 26 May 1977

<sup>c</sup> Evaluated 11 May 1977

<sup>d</sup> Visual estimates of percent composition of weeds in forage at 1st cut.  
Predominant weed species Anthemis cotula and Raphanus sativus.

1953  $\pm$  79 mg. Two treatments, simazine at 1.5 kg/ha (Experiment 6) and diuron at 2.0 kg/ha (Experiment 8), decreased seed weights by 5 percent (Appendix 18). These decreases were not observed at higher rates nor were they consistent between experiments and probably are of no biological significance.

Weed Control. In the field, diuron gave excellent control of many weed species between carbon bands. Weeds that emerged within the carbon band required broadcast herbicide applications. Alfalfa and several other legumes appear to be tolerant to EPTC placed in close proximity to the seed (3). In the greenhouse, EPTC granules seeded with alfalfa resulted in a 99% reduction in the dry weight of perennial ryegrass within the carbon band (Table 7). EPTC retarded ryegrass emergence and those plants that did emerge within the carbon band did not develop beyond the coleoptile stage. Ryegrass outside the carbon band was also retarded by EPTC for a distance of 4 to 5 cm on each side of the row. There was a trend for the high rate of EPTC to reduce the number of alfalfa seedlings and the weight of alfalfa per pot was reduced. Alfalfa injury in the EPTC-treated pots (Table 7) was observed as a cupping of the unifoliolate leaf and sometimes of the first and second trifoliolate leaves. Subsequent leaves showed no injury symptoms. These results suggest that the EPTC granules sown with alfalfa seed may be a satisfactory alternative to broadcast herbicide applications to control grass weeds that develop within the carbon band. Further studies to determine the effectiveness of this technique have been initiated.

Control of Volunteer Legumes. In these experiments, the red clover seeding rate of 4 kg/ha was equivalent to 54 viable seeds per meter of row. At the first seed harvest, an average of 7 to 16 plants per meter remained in the check of trials established on three occasions (Table 3). We expect red clover volunteers in the carbon

TABLE 7. Effect of EPTC granules in the seed row and preemergence diuron on ryegrass control in alfalfa established with carbon bands.<sup>a</sup>

Treatments			Ryegrass						Alfalfa seedlings/pot		
			Within carbon band			Outside carbon band					
			Diuron	EPTC <sup>b</sup>	Ryegrass	Total	1st leaf emerged		Total	1st leaf emerged	
mg.	no.	no.					mg.	no.		no.	
kg/ha	mg/m		mg.	no.	no.	mg.	no.	no.	no.	no.	mg
0	0	-	0	0	0	0	0	0	14	0	497
0	0	+	165	21	20	360	44	44	14	0	393
0	10	+	1	1	0	22	11	5	12	1	409
0	20	+	0	0	0	3	3	0	11	4	297
2	10	+	1	1	0	7	5	1	15	1	502
2	20	+	1	1	0	1	1	0	13	4	375
L. S. D. (0.05)			25	1	1	25	5	3	4	1	92

<sup>a</sup> Harvested at 21 days. Experiment 4.

<sup>b</sup> 10 mg/m EPTC = 2.0 kg/ha in the band, assuming EPTC diffuses to form a band 5 cm spread.

band to also decline because they would be influenced by the same factors that reduced the seeded population, unless the volunteer was a stronger competitor than the red clover seeded as a crop.

Atrazine or diuron at 2 and 3 kg/ha, respectively, controlled 85 to 99% of red clover volunteers (Table 8). Control of volunteers was better in 1977 than 1976. The improved control in 1977 was

Table 8. Effect of three preemergence herbicides on the control of red clover volunteers.<sup>a</sup>

Herbicide	Rate	Control of Volunteers			
		Seedlings, 1976 <sup>b</sup>		- Total <sup>c</sup>	
		Within bands	Between bands	1976	1977
	kg/ha	no. /m	no. /m <sup>2</sup>	— % —	—
Check	0	5	175	0	0
diuron	2.0	6	49	65	93
	3.0	4	9	86	91
	4.0	3	0	95	97
atrazine	1.0	9	19	85	94
	1.5	3	5	94	98
	2.0	2	0	97	99
dichlobenil	4.0	4	62	61	88
	6.0	4	108	46	93
L. S. D. (0.05)		5	37	23	6

<sup>a</sup> Experiment 9.

<sup>b</sup> Evaluated 30 days after sowing.

<sup>c</sup> Visual evaluations 5 (1976) weeks and 25 (1977) weeks after sowing.



associated with a wet soil surface at the time of spraying and the later date of evaluation. Further reductions in the volunteer population in the band should occur with time.

In another experiment, where seedlings were established without carbon bands, diuron, simazine, and atrazine at the lowest rates tested killed all red clover seedlings but not all alfalfa seedlings (Table 2). Control of red clover volunteers in alfalfa should be feasible. However, the control of alfalfa volunteers in red clover may result in seed yield reductions at the first harvest (Table 2).

### CONCLUSION

Under the conditions of these experiments, red clover and alfalfa were successfully established with carbon bands, and diuron at rates from 2.0 to 4.0 kg/ha. Higher rates of diuron may reduce red clover yields when soils are wet on the surface. Control of 85 to 99% of red clover volunteers can be expected with atrazine or diuron at 2.0 and 3.0 kg/ha, respectively.

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## APPENDICES

APPENDIX 1. Effect of preemergence herbicides on white clover seedling counts (Expr. 1).

Herbicide	Rate	I				II				Avg
		R1	R2	R3	Avg	R1	R2	R3	Avg	I + II
	kg/ha	% Control								
Check	0	0	0	0	0	0	0	0	0	0
diuron	0.5	93	100	40	78	100	92	100	97	88
	1.0	100	100	100	100	100	100	100	100	100
	1.5	100	100	100	100	100	100	100	100	100
atrazine	0.5	100	100	100	100	100	100	100	100	100
	1.0	100	100	100	100	100	100	100	100	100
	1.5	100	100	100	100	100	100	100	100	100
simazine	0.5	100	100	100	100	100	100	100	100	100
	1.0	100	100	100	100	100	100	100	100	100
	1.5	100	100	100	100	100	100	100	100	100
bromacil	0.5	100	100	100	100	100	100	100	100	100
	1.0	100	100	100	100	100	100	100	100	100
	1.5	100	100	100	100	100	100	100	100	100

APPENDIX 2. Seedling counts of greenhouse-established white clover (Expr. 3).

Carbon	diuron, kg/ha				
	0	0.5	1.0	2.0	4.0
kg/ha	seedlings per pot				
0	15	1	0	0	0
150	17	14	15	13	8
300	16	16	16	13	13
L.S.D.(0.05)	4.5				

APPENDIX 3. Effect of different herbicides on white clover established with activated carbon bands.<sup>1</sup>

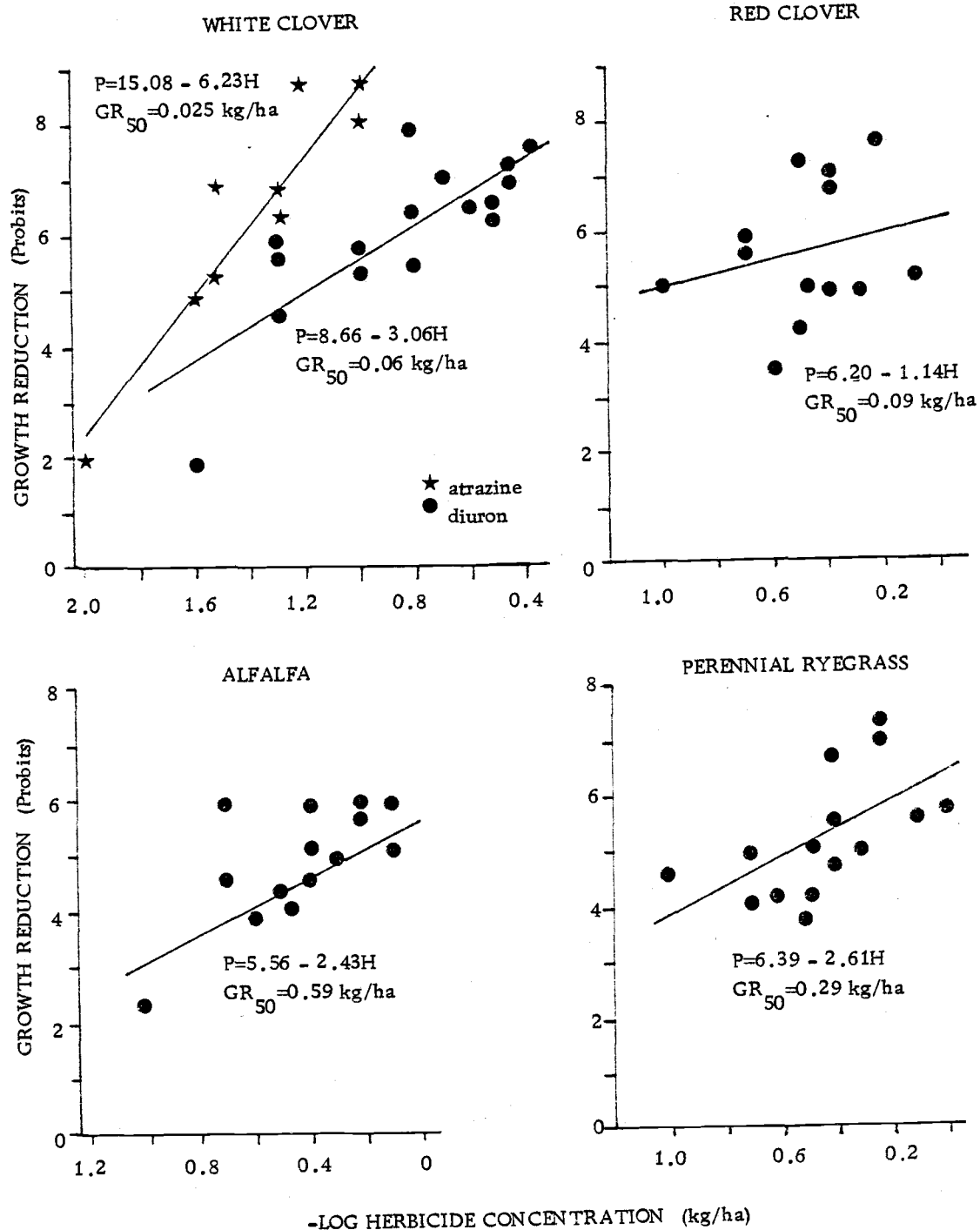
Herbicide	Rate	Seedling Counts <sup>2</sup>		Seed Yield		D.M. Forage Yield	Ground Cover	
		With Carbon	Without Carbon	1977	1978	1978	1977	1978
		kg/ha	Seedlings/m	kg/ha			%	
diuron	2.0	16	0	136	344	2,916	92	100
	3.0	14	0	79	355	2,572	64	100
	4.0	7	0	14	323	1,595	16	97
simazine	1.5	15	0	52	357	2,661	40	100
	2.5	10	0	18	336	1,658	13	96
atrazine	0.75	21	0	138	349	2,825	81	100
	1.25	15	0	72	377	2,972	66	100
2,4-DB	1.2	29	17	235	362	2,776	100	100
Check	0	27	16	169	329	2,518	100	100
L.S.D.(0.05)		5		55	NS	770	21	NS

<sup>1</sup> Experiment 7, established 16 September 1976.

<sup>2</sup> Evaluated 30 December 1976.

APPENDIX 4. White clover seedling numbers with 'Gro-Safe' activated carbon and three herbicides applied preemergence at 0.5 kg/ha (Expr. 4).

Herbicide	Carbon:Herbicide Ratio	I						II						Avg	
		R1	R2	R3	R4	R5	Avg	R1	R2	R3	R4	R5	Avg	I + II	
		seedling numbers/pot													
Check	0	20	21	19	22	22	20.8	7	14	12	15	11	11.8	16.3	
diuron	100:1	25	19	15	23	25	20.8	8	16	2	12	3	8.2	14.5	
	50:1	25	18	16	25	22	21.2	9	9	4	7	13	8.4	14.8	
	25:1	23	19	13	16	6	15.4	0	0	0	0	0	0	7.7*	
atrazine	300:1	16	21	17	19	16	17.8	5	13	3	15	8	8.8	13.3	
	200:1	21	19	19	20	25	20.8	12	6	11	9	6	8.8	14.8	
	100:1	4	0	1	1	0	1.2	10	7	0	0	0	3.4	2.4*	
simazine	200:1	12	19	19	14	24	17.6	3	13	8	11	9	8.8	13.2	
	100:1	13	14	16	16	7	13.2	9	12	3	2	16	8.4	10.7*	
	50:1	11	9	2	6	3	6.2	2	5	5	0	6	3.6	4.9*	
L.S.D.(0.05)							4.8							5.1	4.1



APPENDIX 5. The effect of diuron and atrazine on the topgrowth of four species.  
 $H$  =  $-\log$  herbicide concentration.  $P$  = growth reduction in probit units.  
 When  $P = 5$ , the topgrowth has been reduced by 50% ( $GR_{50}$ ).

APPENDIX 6. White clover seedling numbers 14 weeks after establishment with activated carbon.<sup>1,2</sup>

diuron	Seeding rate, kg/ha			Carbon rate, kg/ha					
	Fall, 1976 <sup>3</sup>			Fall, 1976 <sup>4</sup>			Spring, 1977 <sup>4</sup>		
	2	4	22	200	300	400	200	300	400
kg/ha	seedlings/m of row								
0	17	33	32	44	45	33	26	26	32
2.0	9	25	26	17	29	25	21	18	17
3.0	8	15	19	3	21	15	12	11	9
4.0	6	8	10	1	8	8	5	2	5
L.S.D.(0.05)	5	6	4	9	9	6	4	7	5

<sup>1</sup> Row width = 30 cm. Seedlings/m<sup>2</sup> = seedlings x  $\frac{100 \text{ cm}}{30 \text{ cm}}$

<sup>2</sup> Seedling counts for Experiment 8 are listed in Appendix 3.

<sup>3</sup> Experiment 10, established September 1976.

<sup>4</sup> Experiment 11, established September 1976 and April 1977.

APPENDIX 7. Seed and forage yields and ground cover of white clover established in spring, 1976, with carbon bands and diuron (Expr. 7).

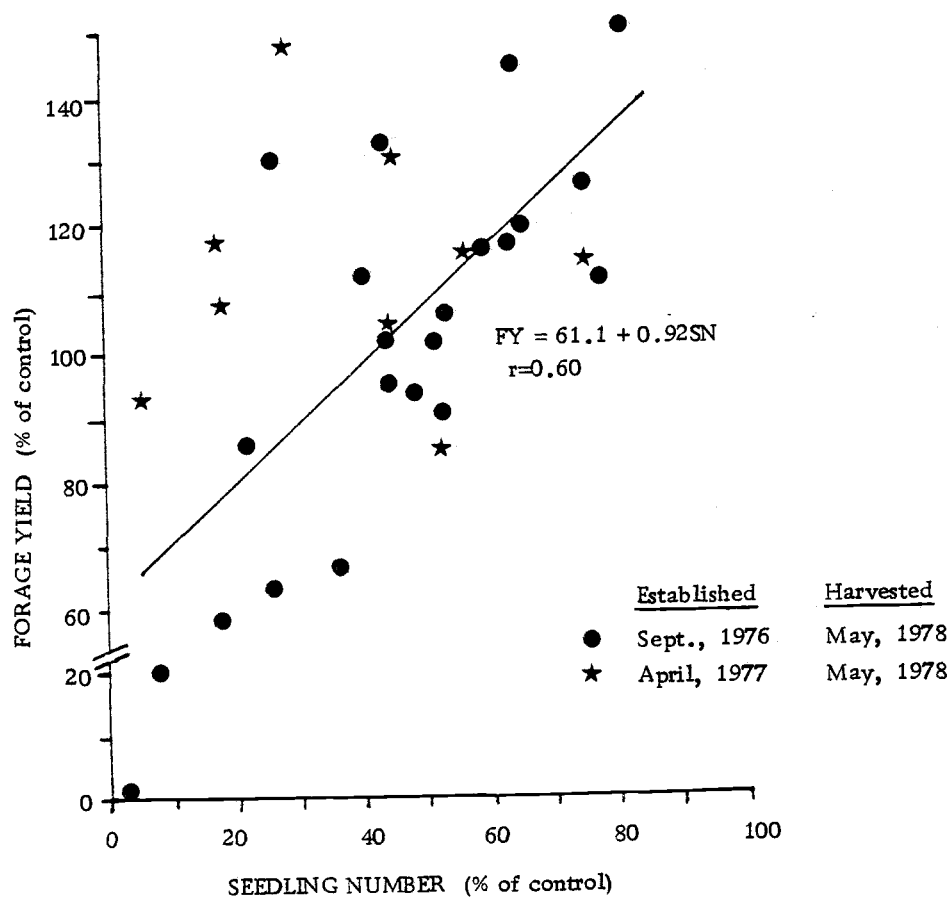
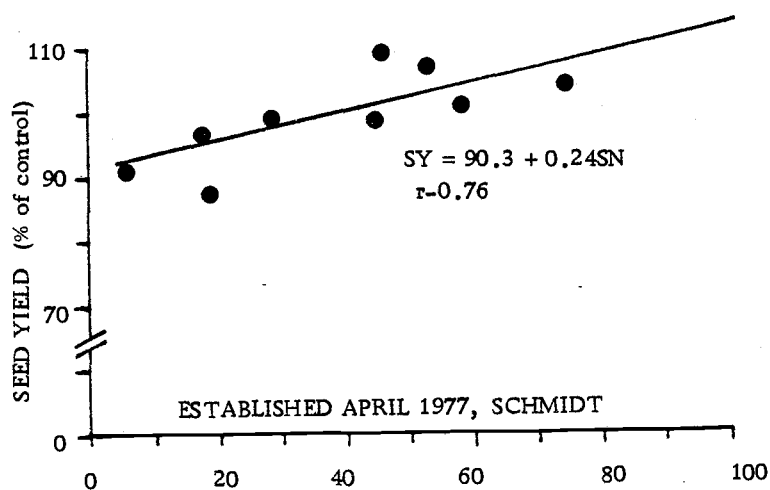
Diuron	Seed Yield		Forage DW		Ground Cover	
	1977	1978	1977	1978	1977	1978
kg/ha	kg/ha		kg/ha		%	
0	401	300	1,089	2,195	100	100
2.0	455	285	999	1,919	100	100
4.0	298	287	366	1,801	81	100
L.S.D.(0.05)	NS	NS	163	690	19	NS



APPENDIX 8. Second harvest seed yields of white clover established in Fall 1976.<sup>1</sup>

	Seeding rate, kg/ha			Row width, cm			Carbon rate, kg/ha		
	30	40	50	2	4	22	200	300	400
diuron									
	kg/ha								
0	367	384	415	349	416	369	390	376	416
2.0	354	409	354	448	370	399	368	368	370
3.0	383	415	364	379	382	365	108	359	382
4.0	332	323	268	394	347	374	6	255	347
L.S.D.(0.05)	NS	88	116	NS	NS	NS	62	74	NS

<sup>1</sup> Experiments 9, 10, and 11 established September 1976, harvested July 1978.



APPENDIX 9. Relation between seedling number (SN) 14 weeks after establishment and seed yield (SY) and forage yield (FY) of white clover.

APPENDIX 10. Forage DW yields for white clover established at different row spaces, seeding rates and activated carbon rates  
(Experiments 9, 10, and 11).

Trial Site	Diuron	Row spacing, cm			Seeding rate, kg/ha			Carbon rate, kg/ha		
		30	40	50	2	4	22	200	300	400
	kg/ha				kg/ha					
Hyslop	0	2281	2018	3028	2741	2132	1920	2152	2397	2132
	2.0	2979	2778	2916	2474	2687	2907	2412	2879	2687
	3.0	2668	2260	2522	2818	2835	2787	439*	2309	2835
	4.0	2525	1319*	1306*	2584	1805	2501	0*	1386	1805
	L.S.D.(0.05)	NS	663	913	NS	830	757	487	1018	830
Schmidt	0	1321	1713	1781	1320	1134	2399	1836	2379	1223
	2.0	1807	1162	1407	1652	625	1778	2089	2744	1022
	3.0	1387	1454	1518	1977	2217	1834	1916	3085	1812
	4.0	1091	1529	1523	828	1236	1177	1970	2216	1434
	L.S.D.(0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

\* Significantly less than check at 5%.

APPENDIX 11. White clover germination percentages at first harvest.

Experiment	Herbicide	Rate	Germination				
			R1	R2	R3	R4	Avg
		kg/ha	%				
7	Check	0	94	96	93	97	95
	diuron	2.0	94	96	97	96	96
		3.0	94	96	97	96	96
		4.0	91	95	96	95	94
	simazine	1.5	93	95	92	96	94
		2.5	91	96	94	95	94
	atrazine	0.75	95	97	96	95	96
		1.25	91	97	95	94	94
	2, 4-DB	1.2	94	95	96	98	96
	L.S.D.(0.05)						2
8	Check	0	96	97	96	96	96
	diuron	2.0	97	93	95	97	95
		4.0	98	94	97	97	97
	L.S.D. (0.05)						3
11	Check	0	94	97	97	94	95
	diuron	2.0	96	97	96	97	96
		3.0	97	97	98	94	96
		4.0	96	94	96	96	95
	L.S.D. (0.05)						2

-----

Average  $\pm$  S. E. for all treatments = 95.3  $\pm$  0.9%.

## APPENDIX 12. White clover seed weights at first harvest.

Experiment	Herbicide	Rate	Weight of 1000 Seeds				
			R1	R2	R3	R4	Avg
		kg/ha	mg				
6	Check	0	657	665	714	657	673
	diuron	2.0	653	675	661	669	666
		3.0	625	653	698	670	661
		4.0	655	668	669	663	664
	simazine	1.5	670	640	650	702	666
		2.5	629	630	647	692	650
	atrazine	0.75	651	658	682	683	669
		1.25	679	670	691	676	679
	2, 4-DB	1.2	652	648	685	674	665
	L.S.D. (0.05)						82
7	Check	0	641	646	626	632	636
	diuron	2.0	643	589	635	641	627
		4.0	632	639	653	693	654
	L.S.D. (0.05)						39
10	Check	0	707	678	665	695	686
	diuron	2.0	672	684	664	693	678
		3.0	683	671	673	650	669
		4.0	648	680	660	673	665
	L.S.D. (0.05)						25

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Average  $\pm$  S.E. for all treatments = 663  $\pm$  15 mg/1000 seeds.

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APPENDIX 13. Red clover plant numbers 36 days after sowing 25 viable seeds per pot.

diuron	Activated Carbon	Red Clover					Survival
		R1	R2	R3	R4	Avg	
kg/ha	kg/ha	no.					%
0	0	11	12	18	21	16	62
	150	19	8	17	13	14	57
	300	20	9	19	21	17	69
1.0	0	0	1	0	4	1	5
	150	14	19	15	17	16	65
	300	20	23	13	21	19	77
2.0	0	2	0	0	1	1	3
	150	14	20	6	13	13	53
	300	12	20	20	17	17	69
4.0	0	1	0	0	5	2	7
	150	19	10	15	19	16	63
	300	16	19	15	5	14	55
L.S.D. (0.05)						6	

APPENDIX 14. Weed percentages in red clover and alfalfa.

Row width	Diuron	Weeds <sup>1</sup>							
		Red clover				Alfalfa			
		R1	R2	R3	Avg	R1	R2	R3	Avg
cm	kg/ha	%				%			
30	0	40	30	15	28	30	40	30	33
	2	22	15	5	14	5	10	7	7
	3	10	15	10	12	3	5	0	3
	4	20	10	7	12	5	7	2	5
	L.S.D.(0.05)				11				4
40	0	10	30	20	20	50	40	30	40
	2	5	7	5	6	7	15	5	9
	3	6	15	5	9	2	4	1	3
	4	2	12	5	7	20	0	2	7
	L.S.D.(0.05)				8				4
80	0	40	40	50	43	40	60	25	42
	2	12	15	5	11	10	10	5	8
	3	15	3	2	7	10	5	1	5
	4	3	0	2	2	3	10	0	5
	L.S.D.(0.05)				12				5

<sup>1</sup> Predominant weed species, mayweed (Anthemis cotula) and wild radish (Raphanus sativus).  
Visual evaluation, May 1977.

APPENDIX 15. First harvest seed yields from red clover established with carbon bands in three seasons at three row spaces and three carbon rates.

Site	Diuron	Row spacing, cm			Carbon rate, kg/ha		
		30	50	80	200	300	400
<hr/>							
<hr/>							
Seed yield, kg/ha							
<hr/>							
Hyslop Fall 1976	0	580	608	452	730	559	612
	2.0	661	574	458	703	525	659
	3.0	657	637	413	701	573	660
	4.0	551	640	316	581	496	471
Schmidt Spring 1977	0	618	518	443	550	461	490
	2.0	584	596	387	529	615	589
	3.0	683	561	371	681	672	441
	4.0	748	544	384	752	518	535
Hyslop Fall 1977	0				933	764	835
	2.0				788	720	795
	3.0				247	314	607
	4.0				40	32	229
Average	-	635	585	403	605	521	577
<u>±</u> SE	-	65	43	48	247	195	162



APPENDIX 16. Alfalfa seedling numbers 40 days after sowing 25 viable seeds per pot.

diuron	Activated carbon	Alfalfa				Survival
		R1	R2	R3	Avg	
kg/ha	kg/ha	no.				%
2.0	0	2	0	2	1	5
	150	17	10	22	16	65
	300	24	10	14	16	64
3.0	0	0	0	0	0	0
	150	22	15	13	17	67
	300	22	18	16	19	75
4.0	0	0	4	3	2	9
	150	22	21	20	21	84
	300	6	24	20	17	67
L.S.D. (0.05)					8	

APPENDIX 17. Red clover germination percentages at first harvest.

Experiment No.	Herbicide	Rate	Germination				
			R1	R2	R3	R4	Avg
		kg/ha	%				
5	Check	0	95	96	96	97	96
	diuron	2.0	95	96	95	97	96
		4.0	97	97	94	97	96
	L.S.D. (0.05)						NS
6	Check	0	94	96	89	91	92
	diuron	2.0	89	92	88	89	90
		3.0	91	94	97	93	94
		4.0	91	89	95	95	93
	simazine	1.5	89	93	92	89	91
		2.5	89	90	94	94	92
	atrazine	0.75	92	94	93	92	93
		1.25	91	91	91	90	91
	2, 4-DB	1.2	96	94	94	90	94
	L.S.D. (0.05)						NS
8	Check	0	93	93	96	93	94
	diuron	2.0	93	93	92	92	93
		4.0	91	92	92	94	93
	L.S.D. (0.05)						NS

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Average  $\pm$  S.E. for all treatments = 93.1  $\pm$  1.9%

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APPENDIX 19. White clover seed yields from plots established in spring with carbon bands and diuron.<sup>1</sup>

diuron	Harvest Date											
	July 1977						July 1978					
	R1	R2	R3	R4	R5	Avg	R1	R2	R3	R4	R5	Avg
kg/ha	Seed yield, kg/ha											
0	406	340	428	533	297	401	279	312	310	250	344	300
2.0	425	420	458	303	668	455	185	336	312	239	353	285
4.0	250	375	428	304	134	298	289	314	281	184	366	287
L.S.D. (0.05)	NS						NS					
C.V. %	34.9						11.9					

<sup>1</sup> Experiment 7, established May 1976 with 330 kg/ha carbon in a 2.5-cm wide band.

APPENDIX 20. White clover seed yields from fall plots established with carbon bands and four herbicides.<sup>1</sup>

Herbicide	Rate	Harvest Date									
		July 1977					July 1978				
		R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
	kg/ha	Seed yield, kg/ha									
Check	0	136	178	179	182	169	428	314	331	244	329
diuron	2.0	85	78	137	244	136	391	358	305	320	344
	3.0	39	66	112	98	79	414	325	333	347	355
	4.0	26	2	24	4	14	312	360	329	294	323
simazine	1.5	31	44	96	35	52	371	397	368	290	357
	2.5	20	13	32	7	18	368	437	351	192	336
atrazine	0.75	180	81	162	128	138	391	364	329	309	349
	1.25	54	126	63	44	72	415	395	375	323	377
2, 4-D	1.2	207	202	232	298	235	406	397	333	314	362
L.S.D.(0.05)		55.1					NS				
C.V. %		37.3					10.9				

<sup>1</sup> Experiment 8 established September 1976 with 400 kg/ha carbon in a 3-cm wide band.

APPENDIX 21. White clover seed yields from fall and spring plots established with carbon bands.<sup>1</sup>

Established	Harvested	diuron	Row width, cm														
			30					40					50				
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
		kg/ha	Seed yield, kg/ha														
<u>Hyslop</u> Sept. 1976	July 1977	0	624	572	587	545	582	334	486	575	545	485	552	445	454	324	443
		2.0	447	557	597	328	482	368	368	326	298	340	480	407	314	199	357
		3.0	251	299	240	147	234	44	97	90	86	79	136	57	86	109	97
		4.0	164	120	111	77	118	81	99	20	11	53	29	16	52	18	29
	L.S.D. (0.05)		92					106					105				
	C.V. %		15.9					27.3					27.9				
	July 1978	0	386	382	310	388	367	434	336	375	390	384	404	349	401	505	415
		2.0	384	412	279	360	359	397	380	395	465	409	428	454	259	318	365
		3.0	419	357	388	369	383	437	404	373	445	415	403	375	314	366	364
		4.0	277	390	325	335	332	349	414	170	357	323	322	279	309	162	268
	L.S.D. (0.05)		NS					NS					NS				
	C.V. %		11.4					14.6					20.6				
<u>Schmidt</u> Apr. 1977	July 1978	0	366	551	447	293	414	401	439	403	458	425	437	445	577	425	471
		2.0	450	522	509	380	465	327	257	417	502	378	518	540	552	364	494
		3.0	476	410	437	456	445	430	368	349	566	428	432	307	382	588	428
		4.0	388	540	540	366	460	420	312	278	507	380	476	377	509	417	445
	L.S.D. (0.05)		NS					NS					NS				
	C.V. %		14.0					15.0					19.9				

<sup>1</sup> Experiment 9, established with 400 kg/ha carbon in a 3-cm wide band.

APPENDIX 22. White clover seed yields from fall and spring plots established with carbon bands.<sup>1</sup>

Established	Harvested	diuron kg/ha	Seeding rate, kg/ha																							
			2.0					4.0					22.0													
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg									
			Seeding rate, kg/ha																							
<u>Hyslop</u> Sept. 1976	July 1978	0	620	360	499	534	503	447	407	486	502	461	421	477	343	567	452									
		2.0	278	430	347	441	374	256	416	432	378	371	542	490	489	466	471									
		3.0	161	312	416	127	254	75	203	111	127	129	380	396	428	480	421									
		4.0	177	140	114	120	138	18	93	88	26	56	147	100	154	129	132									
	L.S.D.(0.05)						173										85					93				
	C.V. %						32.4										18.8					15.4				
	July 1978	0	357	369	397	272	349	380	340	461	483	416	360	272	353	491	369									
		2.0	478	422	373	460	448	329	391	395	364	370	312	401	375	507	399									
		3.0	391	380	340	403	379	384	375	325	445	382	298	353	399	410	365									
		4.0	355	422	423	378	394	187	364	463	373	347	287	423	349	439	374									
	L.S.D.(0.05)						NS										NS					NS				
	C.V. %						11.7										7.5					13.0				
<u>Schmidt</u> Apr. 1977	July 1978	0	434	482	415	452	447	584	518	540	516	540	307	494	489	561	463									
		2.0	452	511	406	478	461	286	428	316	516	461	573	346	535	518	493									
		3.0	540	404	386	390	430	368	349	482	548	437	535	428	467	423	463									
		4.0	414	380	463	307	391	474	425	559	436	474	485	417	515	391	452									
	L.S.D.(0.05)						NS										NS					NS				
	C.V. %						13.4										18.0					18.9				

<sup>1</sup> Experiment 10, established with 400 kg/ha carbon in a 3-cm wide band.

APPENDIX 23. White clover seed yields from fall and spring plots established with carbon bands.<sup>1</sup>

Established	Harvested	diuron kg/ha	Carbon rate, kg/ha														
			200					300					400				
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
			Seed yield, kg/ha														
<u>Hyslop</u> Sept. 1976	July 1977	0	448	920	568	441	594	473	459	477	496	476	447	407	486	502	461
		2.0	206	127	322	349	251	398	390	301	421	376	256	416	432	378	371
		3.0	2	4	0	30	9	317	120	274	281	248	75	203	111	127	129
		4.0	0	0	0	0	0	50	10	34	61	39	18	93	88	26	56
	L.S.D. (0.05)	216					76					76					
	C.V. %	31.6					16.6					18.8					
	July 1978	0	314	445	425	373	390	311	421	399	373	376	380	340	461	483	416
		2.0	351	375	331	415	368	351	434	309	375	368	329	391	395	364	370
		3.0	28	169	88	156	108	377	373	386	301	359	384	375	325	445	382
		4.0	6	4	15	0	6	232	221	272	296	255	187	364	463	373	347
	L.S.D. (0.05)	63					NS					NS					
	C.V. %	18.2					13.6					17.5					
<u>Schmidt</u> April 1977	July 1978	0	526	540	397	502	490	447	465	253	275	360	491	415	426	472	452
		2.0	569	393	559	537	515	403	323	399	423	388	528	539	439	428	483
		3.0	417	545	535	447	483	524	342	425	399	423	344	423	460	557	447
		4.0	460	482	369	406	430	299	379	377	351	351	498	426	448	386	439
	L.S.D. (0.05)	NS					NS					NS					
	C.V. %	15.6					20.8					15.2					

APPENDIX 24. Red clover seed yields from plots established in spring with carbon bands and diuron.<sup>1</sup>

diuron	Harvest Date											
	October 1976						August 1977					
	R1	R2	R3	R4	R5	Avg	R1	R2	R3	R4	R5	Avg
kg/ha	Seed yield, kg/ha											
0	403	268	478	739	546	487	354	387	427	515	435	424
2.0	424	533	628	577	711	575	390	409	400	388	356	387
4.0	104	106	137	377	405	226	137	157	212	311	339	231
L.S.D.(0.05)	106											
C.V. %	21.1						16.2					

<sup>1</sup> Experiment 5, established May 1976 with 330 kg/ha carbon in a 2.5-cm wide band.

APPENDIX 25. Red clover seed yields from fall plots established with carbon bands and four herbicides.<sup>1</sup>

Herbicide	Rate	Harvest Date									
		August 1977					August 1978				
		R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
	kg/ha	Seed yield, kg/ha									
Check	0	452	470	730	696	587	594	566	548	591	575
diuron	2.0	347	483	484	499	453	458	562	526	572	530
	3.0	354	277	455	618	426	551	439	471	612	518
	4.0	148	176	514	515	338	498	502	518	632	538
simazine	1.5	218	161	372	521	318	575	397	656	573	550
	2.5	54	39	331	336	190	524	542	636	430	533
atrazine	0.75	208	196	297	667	342	470	590	570	678	577
	1.25	204	111	531	660	377	516	487	487	544	508
2,4-DB	1.2	466	489	712	682	587	582	610	588	808	647
L.S.D. (0.05)		127					NS				
C.V. %		22.8					12.7				

<sup>1</sup> Experiment 6, established September 1976 with 400 kg/ha carbon in a 3-cm wide band.



APPENDIX 26. Red clover seed yields from fall and spring plots established with carbon at three row widths.<sup>1</sup>

Established	Harvested	diuron kg/ha	Row width, cm														
			30					50					80				
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
			Seed yield, kg/ha														
<u>Hyslop</u> Sept. 1976	August 1977	0	678	600	507	533	580	644	689	561	540	608	359	574	466	412	452
		2.0	507	673	717	747	661	541	750	521	484	574	372	509	525	424	458
		3.0	668	683	569	709	657	698	606	541	702	637	473	517	365	298	413
		4.0	577	499	621	509	551	811	637	585	528	640	217	395	396	259	316
	L.S.D. (0.05)		NS					NS					NS				
	C.V. %		14.6					14.2					16.2				
	August 1978	0	504	546	636	552	560	568	584	514	588	564	444	424	552	350	443
		2.0	522	578	572	530	550	538	494	548	436	504	390	406	408	344	387
		3.0	586	498	560	380	506	472	608	532	450	515	356	318	410	398	371
		4.0	576	604	390	438	487	538	524	524	602	547	446	368	454	266	384
	L.S.D. (0.05)		NS					NS					NS				
	C.V. %		13.9					10.5					12.7				
<u>Schmidt</u> April 1977	August 1978	0	640	550	698	686	618	598	268	756	448	518	542	374	382	416	404
		2.0	430	686	692	538	584	618	678	514	572	596	626	266	220	316	357
		3.0	640	544	794	654	683	670	472	552	550	561	500	236	400	242	354
		4.0	650	870	596	876	748	554	570	534	516	544	270	276	278	186	268
	L.S.D. (0.05)		NS					NS					NS				
	C.V. %		19.9					32.7					25.3				

<sup>1</sup> Experiment 7, established with 400 kg/ha carbon in bands 3-cm wide.

APPENDIX 27. Red clover seed yields from fall and spring plots established with carbon bands.<sup>1</sup>

Established	Harvested	diuron kg/ha	Carbon rate, kg/ha															
			200					300					400					
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	
			Seed yield, kg/ha															
<u>Hyslop</u> Sept. 1976	August 1977	0	626	709	631	957	730	535	460	569	670	559	589	594	580	688	612	
		2.0	662	610	787	755	703	571	462	481	586	525	657	496	807	675	659	
		3.0	517	597	928	762	701	590	471	654	576	573	659	586	670	724	660	
		4.0	480	616	682	548	581	515	465	420	582	496	434	375	623	453	471	
	L.S.D. (0.05)		NS					NS										112
	C.V. %		16.4					10.1										11.7
	August 1978	0	604	594	636	632	617	394	594	480	662	533	536	524	540	556	539	
		2.0	506	480	630	554	543	426	416	618	588	512	530	590	486	550	539	
		3.0	588	630	620	646	621	386	490	622	520	505	552	534	536	462	521	
		4.0	576	586	480	520	540	590	554	418	344	477	510	376	324	538	437	
	L.S.D. (0.05)		NS					NS										NS
	C.V. %		8.5					23.3										12.1
<u>Schmidt</u> April 1977	August 1978	0	416	562	614	606	550	552	388	390	562	461	480	550	378	550	490	
		2.0	508	430	550	628	529	736	632	484	608	615	388	660	714	596	589	
		3.0	596	752	656	722	681	744	528	640	778	672	560	358	462	484	441	
		4.0	662	774	792	780	752	486	384	560	642	518	442	436	630	632	535	
	L.S.D. (0.05)		90.1					125										NS
	C.V. %		9.0					13.8										24.1

(continued on next page)

APPENDIX 27. Continued.

Established	Harvested	diuron	Carbon rate, kg/ha														
			200					300					400				
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
		kg/ha	Seed yield, kg/ha														
<u>Hyslop</u>																	
Oct. 1977	August 1978	0	956	976	886	914	933	834	720	726	778	764	814	754	816	956	835
		2.0	886	766	840	660	788	626	542	944	766	720	704	874	808	792	795
		3.0	216	410	140	222	247	594	184	108	368	314	784	604	438	600	607
		4.0	142	18	0	0	40	16	52	32	30	32	490	182	110	134	229
	L.S.D. (0.05)						126					236					200
	C.V. %						15.7					32.4					20.3

<sup>1</sup> Experiment 8, established with 30-cm wide rows and carbon bands 3-cm wide.

APPENDIX 28. Seed yields of fall-established alfalfa.<sup>1</sup>

Row width	Diuron	Seed Yield				
		R1	R2	R3	R4	Avg
	kg/ha	kg/ha				
30 cm	0.0	218	293	288	328	282
	2.0	313	171	278	276	260
	3.0	202	209	262	233	227
	4.0	271	291	300	289	288
	L.S.D. (0.05)	NS				
	C.V. %	15.4				
50 cm	0.0	278	324	356	406	341
	2.0	249	244	369	422	321
	3.0	307	271	386	320	321
	4.0	307	406	531	488	432
	L.S.D. (0.05)	76				
	C.V. %	12.4				
80 cm	0.0	268	375	373	376	348
	2.0	519	417	464	433	458
	3.0	577	346	498	418	460
	4.0	537	384	504	304	432
	L.S.D. (0.05)	NS				
	C.V. %	17.4				

<sup>1</sup> Established 20 September 1976. Harvested 14 September 1977.

APPENDIX 29. Application data for diuron and other soil active herbicides applied preemergence to legumes established with carbon bands.

	White Clover			Alfalfa	
	Hyslop	Hyslop	Schmidt	Hyslop	Schmidt
Location					
Date	13 May 1976	20 Sept. 1976	2 Apr. 1977	20 Sept. 1976	25 Mar. 1977
Experiment	7	8, 9, 10, 11	9, 10, 11	6, 7	7
Conditions:					
Air temperature (C)	21	24	16	24	9
Soil temperature, 5 cm (C)	23	22	16	22	8
Relative humidity	65	-	52	-	70
Cloud cover (%)	80	10	25	10	60
Wind speed (km/h)	13	7	8	7	5
Soil moisture at surface	Dry to 15 mm	Dry to 15 mm	Dry to 5 mm	Dry to 15 mm	Dry to 5 mm
Method of Application	Broadcast	Broadcast	Broadcast	Broadcast	Broadcast
Carrier volume	300	225	225	225	225
Nozzle size	8004	8002	8002	8002	8002
Pressure (k Pa)	195	195	195	195	195
Stage of growth:	Preemergence	Preemergence	Preemergence	Preemergence	Preemergence

APPENDIX 29. Continued

Red Clover				
Location	Hyslop	Hyslop	Schmidt	Hyslop
Date	13 May 1976	20 Sept. 1976	3 Apr. 1977	3 Oct. 1977
Experiment	5	6, 7, 8	7, 8	8
Conditions:				
Air temperature (C)	21	24	16	18
Soil temperature, 5 cm (C)	23	22	17	18
Relative humidity (%)	65	-	53	56
Cloud cover (%)	80	10	65	10
Wind speed (km/h)	13	7	6	6
Soil moisture at surface	Dry to 15 mm	Dry to 15 mm	Dry to 5 mm	Moist on surface
Method of Application	Broadcast	Broadcast	Broadcast	Broadcast
Carrier volume	300	225	225	225
Nozzle size	8004	8002	8002	8002
Pressure (k Pa)	195	195	195	195
Stage of growth:	Preemergence	Preemergence	Preemergence	Preemergence

## APPENDIX 30. Tolerance of three legume species to preemergence herbicides.

### INTRODUCTION

A screening trial was designed to establish whether any of the tested herbicides, applied preemergence, were either selective between species of forage legumes or had potential for use in activated carbon experiments.

### MATERIALS AND METHODS

A greenhouse trial was designed to evaluate the effect of seven herbicides at three rates on legume species, white clover (Trifolium repens L. 'Grasslands Huia'), red clover (Trifolium pratense L. 'Grasslands Hamua'), and big trefoil (Lotus pendunculatus Cav. 'Grasslands Maku'). One hundred viable seeds of each species were sown into a plastic tray (42 x 30 x 7 cm) divided into three equal portions, allocated on a random basis. A 50:50 mixture of sterilized soil: sand with fertilizer and lime were added. The seeds were broadcast-sown and covered to a depth of 5 mm with the soil mixture. The experiment, initiated in June 1974, was a split plot design.

Twenty-four hours after sowing, the soil surface of the trays were sprayed at 480 liters/ha, using a small hand-sprayer. Each tray was sprayed with one herbicide at one rate.

The trays were surface-watered daily. The average daily maximum and minimum temperatures during the experiment were 23.0 and 11.3°C, respectively. Seedling numbers were counted and the stage of development recorded weekly. After six weeks, plant dry weights were measured.

## RESULTS AND DISCUSSION

In the screening trial, both dalapon on all species and ethofumesate on 'Grasslands Maku' lotus, showed some selectivity in terms of seedling survival (Table A30.1), although even these treatments caused yield depressions from 40 to 66% at the lowest rates tested (Tables A30.2 and A30.3). The chlorthiamid treatment inhibited seedling emergence. In a subsequent experiment, chlorthiamid rates equivalent to 3.0 and 1.5 kg/ha also inhibited seedling emergence.

The overall response with time can be described in general terms. Both diuron and amitrole resulted in a sudden and complete collapse of the seedling populations two to four weeks after sowing. Ethofumesate and dalapon resulted in a decrease in the seedling population two weeks after sowing. Chlorthiamid and, to a lesser extent, amiben inhibited or delayed germination.





Table A30.2. Effect of dalapon on legume seedling dry weights 42 days after sowing.

Herbicide	Rate	Species											
		Trifolium repens				T. pratense				L. pedunculatus			
		R1	R2	R3	Avg	R1	R2	R3	Avg	R1	R2	R3	Avg
	kg/ha	mg/plant											
Check	0	8.2	6.8	12.3	9.1	16.2	16.1	17.3	16.5	13.4	11.7	15.1	13.4
dalapon	3.0	3.7	2.6	2.6	3.0	8.7	6.1	8.0	7.6	7.1	7.3	10.0	8.1
	6.0	2.6	2.1	2.6	2.4	5.7	3.4	4.2	4.4	5.8	5.2	5.3	5.4
	12.0	2.0	1.9	2.7	2.2	6.9	4.9	3.8	5.2	4.6	7.5	5.6	5.9

L.S.D. (0.05) = 2.2 mg

Table A30.3. Effect of ethofumesate on 'Grasslands Maku' lotus seedling dry weights 42 days after sowing.

Rate	R1	R2	R3	Avg
kg/ha	mg/plant			
0	13.4	11.7	15.1	13.4
0.5	4.8	4.8	7.6	5.7
1.0	2.9	4.3	4.5	3.9
2.0	2.6	3.1	2.2	2.6

## APPENDIX 31. White clover establishment with carbon bands and preemergence herbicides.

### INTRODUCTION

The tolerance of white clover (Trifolium repens L. 'Grasslands Huia') established with activated carbon and herbicides was studied in the greenhouse and the field.

### MATERIALS AND METHODS

Greenhouse experiments were conducted with 300 kg/ha activated carbon (Gro-Safe) and preemergence herbicides with the same general methods outlined in Chapter III. The field experiments were established at Hyslop on 12 May 1976 using the same general methods and conditions outlined in Chapter III, Experiments 7 and 12. The experiment consisted of two adjacent trials. In one trial, white clover was sown as the crop. In the other trial, white clover was broadcast-sown at a rate of 1400 viable seeds/m<sup>2</sup> and incorporated to a depth of 7 cm. Red clover was then established as a crop with carbon bands in the white clover-contaminated soil. The number of volunteer white clover seedlings between carbon bands were counted 30 days later.

### RESULTS

In the greenhouse, activated carbon protected white clover from low rates of diuron, atrazine, simazine, and atrazine + prometryn (1:1), applied preemergence (Table A31.1). In the field, 165 and 330 kg/ha of activated carbon gave similar levels of protection (Table A31.2). Diuron, atrazine, and atrazine + prometryn (1:1)

at rates up to 3.0, 1.0, and 1.0, respectively, gave adequate protection to white clover with complete or near complete control of volunteer white clover.

Table A31.1 Effect of four preemergence herbicides on white clover seedlings protected by 300 kg/ha of activated carbon.<sup>1,2</sup>

Herbicide	Rate (kg/ha)	(Seedlings/pot)					% Survival
		R1	R2	R3	R4	Avg	
Check	0	11	17	16	15	14.8	59
diuron	2.0	20	14	16	18	17.0	68
atrazine	0.5	22	19	10	8	14.8	59
	1.0	1	14	5	18	9.5	38
	2.0	4	6	0	1	2.8	11
simazine	0.5	14	19	20	19	18.0	72
	1.0	19	21	25	24	22.3	89
	2.0	21	4	11	6	10.5	42
atrazine + prometryn <sup>3</sup> (1:1)	0.5	8	25	3	19	13.8	55
	1.0	9	11	6	4	7.5	30
	2.0	0	0	0	1	0.3	1
L. S. D. (0.05)						7.8	

<sup>1</sup> 25 seeds per pot

<sup>2</sup> Harvested 38 days after sowing

<sup>3</sup> 2,4-bis(isopropylamino)-6-methylthio-s-triazine

Table A31.2. White clover growth scores 15 weeks after planting, and number of volunteer white clover seedlings between carbon bands 30 days after sowing.

Herbicide	Rate	Activated Carbon	Growth Score <sup>1</sup>					Seedlings between carbon bands				
			R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
			kg/ha	kg/ha	Seedlings/1000 cm <sup>2</sup>							
Check	0	165	5	5	5	5	5.0	11	9	16	16	13.0
		330	5	5	5	5	5.0	12	10	13	10	11.2
diuron	2.0	165	4	4	4	4	4.0	0	0	0	0	0.0
		330	4	3	4	4	3.8	0	0	1	0	0.3
	3.0	165	3	1	2	2	2.0	0	0	0	0	0.0
		330	3	1	3	3	2.5	0	0	0	0	0.0
	4.0	165	0	0	0	0	0.0	0	0	0	0	0.0
		330	0	1	1	1	0.8	0	0	0	0	0.0
atrazine	1.0	165	2	2	3	4	2.8	0	0	0	0	0.0
		330	2	3	4	4	3.3	0	0	0	0	0.0
	1.5	165	0	0	1	1	0.5	0	0	0	0	0.0
		330	0	1	1	1	0.8	0	0	0	0	0.0
	2.0	165	0	0	0	1	0.3	0	0	0	0	0.0
		330	1	1	0	1	0.8	0	0	0	0	0.0
dichlobenil	2.0	165	5	5	5	5	5.0	5	5	0	1	2.8
		330	5	5	5	5	5.0	0	1	2	2	1.3
	4.0	165	3	5	5	5	4.5	4	4	3	3	3.5
		330	3	5	5	5	4.5	3	1	7	2	3.3
	6.0	165	5	5	5	5	5.0	2	4	5	2	3.3
		330	5	5	5	5	5.0	2	2	1	2	1.8
atrazine + prometryn (1:1)	1.0	165	3	3	5	5	4.0	0	0	0	0	0.0
		330	4	3	5	5	4.3	4	0	0	0	1.0
	1.75	165	2	1	2	3	2.0	0	0	0	0	0.0
		330	2	2	2	3	2.3	0	1	0	0	0.3
	2.5	165	1	0	1	1	0.8	0	0	0	0	0.0
		330	1	1	1	1	1.0	0	0	0	0	0.0
<sup>1</sup> Score 0-5 Where 0 = no clover present 5 = growth of check plot			L. S. D. (0.05) Carbon 0.6 Herbicides 1.1					L. S. D. (0.05) Herbicides 1.93				

## APPENDIX 32. Depth of emergence of white clover.

## INTRODUCTION

The objective of this experiment was to determine from what depth white clover (Trifolium repens L. 'New Zealand') can emerge from.

## MATERIALS AND METHODS

One hundred seeds per pot were sown at six depths in 10 x 10 x 10-cm pots. Thirty days after sowing, the number of seedlings emerged and the shoot dry weights were determined.

## RESULTS AND DISCUSSION

White clover establishment from a depth of 3 cm or greater was poor (Table A32). If legume hard seed in the soil is concentrated near the surface, it may be possible to deep-plow and bury the potential contaminants.

Table A32. Percent survival and shoot dry weight of white clover seedlings 30 days after sowing at different depths.

Sowing Depth	R1	R2	R3	R4	R5	Avg
cm	<hr/>					
	%					
1.0	52	70	56	57	48	57
2.0	61	57	29	30	34	42
3.0	19	7	9	0	0	7
4.0	3	0	1	0	0	1
5.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
L. S. D. (0.05)						9
	<hr/>					
	mg					
1.0	21.4	18.9	22.7	23.5	28.9	23.1
2.0	18.4	21.1	21.3	21.4	21.9	20.8
3.0	14.7	13.0	10.6	0	0	7.7
4.0	4.0	0	3.0	0	0	1.4
5.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
L. S. D. (0.05)						10.6

## APPENDIX 33. A comparison of three carbon types for carbon-banding systems.

### INTRODUCTION

The objective of this experiment was to compare two new carbon products with an existing carbon product in a carbon-banding system.

### MATERIALS AND METHODS

Greenhouse experiments using the general methods outlined in Chapters III and IV were used. The following two activated carbon products, Gro-Safe (I. C. I. United States, Wilmington, DE 19897) and PA Carb (Pacific Carbon, Inc., P. O. Box 755, Blue Lake, CA 95525) and a non-activated graphite product were tested at 300 kg/ha. Italian ryegrass (Lolium multiflorum Lam.) and red clover (Trifolium pratense L. 'Kenland') were sown at a depth of 10 mm and the carbon applied to the soil surface. Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] was applied preemergence at 0, 2, and 4 kg/ha.

### RESULTS AND DISCUSSION

Both activated carbon products (Gro-Safe and PA Carb) were similar in their ability to protect annual ryegrass and red clover from diuron. The non-activated graphite gave no protection and was toxic to the annual ryegrass (Table A33).



Table A33. Response of annual ryegrass and red clover to preemergence diuron with 300 kg/ha carbon applied before the herbicide treatment.

Carbon	diuron	Annual ryegrass shoot dry weight/pot					Red clover shoot dry weight/pot				
		R1	R2	R3	R4	Avg	R1	R2	R3	R4	Avg
	kg/ha	mg									
--	0	1868	1694	2526	2010	2024	1645	2489	2063	2272	2117
	2.0	196	77	208	185	167	0	1180	0	0	295
	4.0	0	224	0	0	56	0	123	978	0	275
Gro-Safe	0	1419	1678	1853	1841	1698	2540	2582	2070	2630	2456
	2.0	1676	1635	2114	2175	1900	2078	2825	1668	1284	1964
	4.0	2306	1674	2313	2170	2116	1643	2315	2037	2542	2134
P.A. Carb	0	1696	1560	2003	2282	1885	1540	1700	2364	2364	1992
	2.0	2080	1831	2023	2104	2010	2241	1213	2162	2773	2097
	4.0	1705	1782	2413	2213	2028	1174	1587	1362	1373	1374
Graphite	0	1224	190	49	1107	643	2456	2355	1450	1676	1984
	2.0	73	0	0	0	18	0	0	0	0	0
	4.0	0	0	155	19	44	715	0	0	0	179
L.S.D. (0.05)						320					

## APPENDIX 34. Carbon and non-carbon banding with diuron.

## INTRODUCTION

The objective of this experiment was to compare the effectiveness of a physical barrier, that prevents herbicide being placed above the seed row, with a carbon band, in preventing white clover injury to diuron.

## MATERIALS AND METHODS

Two greenhouse experiments using 10 x 10 cm pots were conducted using 'Grasslands Huia' white clover. Angle iron strips were laid over the seed row to give non-carbon bands 2.5 and 4.0 cm in width. Pots were subsurface-irrigated and evaluated 42 days after the post-plant, preemergence applications of diuron. Each experiment was a randomized complete block design with five replications per treatment.

## RESULTS AND DISCUSSION

Seedling survival following diuron treatments were similar in both carbon and non-carbon bands (Table A34), although there was a trend for carbon bands to give greater protection. Shoot dry weights per seedling were not reduced by either of the protection methods.

## CONCLUSION

The results of this greenhouse study suggest that non-carbon bands may be an alternative to carbon banding, although lateral

movement of the herbicide following rain may reduce the effectiveness of this method or protection.

Table A34. White clover seedling plant numbers as a percentage of seeds sown and shoot dry weights when carbon and non-carbon bands are used to increase selectivity of a pre-emergence application of diuron.

Carbon	Precision band width	diuron	Plant numbers surviving		Shoot DW
kg/ha	cm	kg/ha	%	$\arcsin \sqrt{x}$	mg
0	-	0.0	67	56	96
0	-	1.5	0*	0	0
0	-	3.0	0*	0	0
0	2.5	1.5	36*	35	108
0	4.0	1.5	53	47	130
0	2.5	3.0	46	43	107
0	4.0	3.0	50	48	117
300	-	0.0	76	68	104
300	-	1.5	66	56	113
300	-	3.0	60	53	97
L. S. D. (0.05)			-	15	44

\* Significantly different from check at L. S. D. (0.05).