A STUDY OF VARIATION AND INHERITANCE

OF RESISTANCE TO <u>FUSARIUM</u> ROOT ROT

IN RED CLOVER (<u>TRIFOLIUM</u> <u>PRATENSE</u> L.)

bу

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#### ABSTRACT

In a greenhouse study, eight month old plants of red clover cys Arlington and Florex were evaluated for resistance to Fusarium root rot. Four hundred and fifty genotypes were inoculated with one of three isolates of Fusarium roseum (814, 927, 959) using a cut-root application technique. The degree of Fusarium root rot incidence was evaluated by measuring the vertical discoloration in the taproot from the point of inoculation, and by means of the Horsfall-Barratt scale. Isolate 814 produced a greater degree of infection than isolates 927 and 959. Genetic difference in resistance between and within the two red cultivars were found using isolate 814 and 959, but no difference was found using the less pathogenic isolate 927.

Crosses were made between and among genotypes found resistant (R), and susceptible (S) to isolate 814 and their progenies were screened for resistance to this isolate. The proportion of resistant, intermediate and susceptible genotypes obtained in the progeny was similar to their parents, when the two cultivar tested were pooled together. However, opposite results were found when comparing the results obtained within each cultivar. In Florex, progenies of R x R crosses generally showed a greater degree of resistance than progenies of S x S crosses. In Arlington, progenies of S x S crosses showed a lesser degree of resistance than the R x R crosses. Narrow-sense heritability was estimated at 37% when calculated over the Florex cultivar only.

About 40% of the plants tested showed some internal breakdown. The enlargement of the crown was positively correlated with the incidence of internal breakdown, as well as the occurence of <u>Fusarium</u> root rot severity, particularly in Arlington. Decrease in yield was correlated with the increase of <u>Fusarium</u> root rot as well as the occurence of internal breakdown.

Dans une étude en serre, des plants de trêfle rouge ont été évalués quant à leur résistance au pourridié fusarien. Ces plants âgés de huit mois provenaient des cultivars Arlington et Florex. Au total, quatre cent cinquante (450) génotypes ont été inoculés avec l'une des trois races de <u>Fusarium roseum</u> (814, 927 et 959) en utilisant une technique d'application directe du champignon sur les racines coupées. Le degré de sensibilité au champignon a été évalué en mesurant la hauteur d'infection des tissus de la racine pivotante à partir du point d'inoculation, et par l'usage de l'échelle Horsfall-Barratt. La race 814 a provoqué plus d'infection que les races 927 ou 959. Des différences génétiques ont été trouvées entre, et à l'intérieur des deux cultivars de trêfle rouge, avec les races 814 et 959. Aucune différence n'a été observée pour la race 927, la moins virulente.

Des croisements ont été effectués entre les génotypes classés résistants (R) et sensibles (S) à la race 814 et les descendants furent à leur tour évalués pour leur sensibilité à cette race. La proportion de plantes résistantes, intermédiaires et sensibles obtenue pour la première génération s'est avérée similaire à celle des parents, lorsque les résultats des deux cultivars sont amalgamés. Cependant, des résultats opposés ont été observés lorsqu'on les examine dans chacun des cultivars. Chez Florex, les descendants des croisements entre plantes résistantes (R x R) démontrent une plus grande résistance au pourridié fusarien que les descendants provenant de croisements entre des plantes sensibles (S x S). Par contre, chez Arlington, les descendants provenants de croisements entre des plantes sensibles demontrent un niveau de résistance plus élevé que les plantes provenant de croisements entre des plantes classifiées résistantes (R x R). Une héritabilité restreinte de 37%, de la sensibilité au Fusarium, a été obtenue chez le cultivar Florex.

On a découvert la présence d'une desintégration interne de la racine chez 40% des plants de la population des parents. Cette affection d'ordre physiologique survenait plus fréquemment dans les grosses racines et a été associée, surtout chez Arlington, à la sensibilité au pourridié fusarien. Une baisse du rendement en matière sèche a été associée à la sensibilité au pourridié ainsi qu'à la présence de la désintégration interne de la couronne.

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#### I. INTRODUCTION

Red clover represents one of the most important legumes grown in North America as well as in most European countries, New Zealand and Australia. The 1981 Census figures from Statistics Canada revealed that over 44% of the 1929 tonnes of legume seeds sold in the Province of Quebec were red clover.

Red clover production in the Province of Quebec started with the beginning of the colony. Its popularity grew with the increasing number of European settlers. The majority of Quebec producers seed their hay fields with a mixture of red clover and timothy because this is what their parents were using (Lambert, 1981). Until recently, few farmers were familiar with the requirements of this species to obtain maximum production.

Alfalfa has been suggested as a better alternative to red clover. Alfalfa has been shown to yield more than red clover under the climatic conditions prevailing in the Province of Quebec, if properly managed. Indeed, alfalfa requires plentiful available calcium, phosporus and potassium for proper establishment and growth, and often, micronutrients such as boron mean the difference between success and failure (Heath et al., 1973). Good drainage is essential to its winter survival and subsequent performance. Therefore, producing alfalfa under the environmental conditions prevailing in Quebec is quite expensive. It requires the modification of prevailing soil conditions by drainage, liming and adequate fertilization.

The main advantage of alfalfa over red clover is its longer survival under field conditions. An alfalfa stand can persist and produce an

economic forage yield for 5 to 6 years, while a red clover field rarely lasts longer than 2 to 3 years. Botanically, red clover is also a perennial plant which can persist more than four years under favourable conditions (Fergus and Hollowell, 1960; Gasser and Gagnon, 1976).

Why then does red clover, a perennial legume, not persist longer under field conditions? Several studies have been conducted to determine the factors involved. The main reasons found so far are: (a) improper management, (b) adverse environmental factors and (c) the susceptibility of red clover to diseases, particularly the crown and root rot disease complex (Leath et al., 1971).

Therefore, if a new cultivar of red clover could be developed which would persist longer, it would be of major significance to Quebec, and Northeastern American agriculture. Less investment would be required to produce high yields of a nutritious forage than are presently needed, and less costly modifications would be required to accomodate more demanding forage legumes. Moreover, we should take care to maintain a high quality legume alternative to alfalfa. The culture of alfalfa is relatively new in Quebec, and deleterious insects and diseases might not have had enough time to show their aggressiveness on this crop as is the case on red clover.

Several approaches are possible to improve the persistence of red clover. Selection for genotypes resistant to <u>Fusarium</u> root rot is one of them, notwithstanding the difficulties associated with this procedure (Dijkstra, 1964; Leath <u>et al</u>., 1971.).

Very little is known about the genetic transmission of <u>Fusarium</u> root rot resistance in red clover (Leath <u>et al</u>., 1971). In 1980, Richard <u>et al</u>. demonstrated that genetic selection against <u>Fusarium</u> root rot was possible in an alfalfa population, even if its heritability was quite

- low. Sustained with the hope the same results could be achieved with a different scheme of breeding procedures, it was decided to study the reaction of two cultivars of red clover to selection for <u>Fusarium</u> root rot resistance. The objectives of our study were as follows:
  - a. Assess the virulence of three <u>Fusarium</u> isolates on two red clover cultivars
  - b. Identify genotypes with high levels of resistance to <u>Fusarium</u>
    root rot
  - c. Determine the inheritance of resistance to Fusarium root rot.

#### II. LITERATURE REVIEW

#### II.1 RED CLOVER DISEASES

Red clover (<u>Trifolium pratense L.</u>) is affected by quite a wide range of diseases. Nyvall (1979) reports on 23 diseases of red clover. The names of these diseases, their causal agents and their distributions are summarized in Table 1.

Agriculture Quebec (1975) published a report where forty-six agents were listed as causing a reduction of the lifespan of red clover crops.

Among these, there are 28 fungi, 8 viruses, 5 nematodes, 2 mycoplasma, 2 mineral deficiencies and one bacterium (Tetteh, 1980).

According to Fergus and Hollowell (1960), disease attack is responsible for approximately 50 percent of the economic losses incurred in red clover. Among these diseases, the root and crown diseases are considered as the most important ones (Hanson and Kreitlow, 1954; Chi, 1965; Leath et al., 1971).

Breeding for disease resistance in red clover therefore appears to be economically worthwhile.

# II.2 DISEASE RESISTANCE IN RED CLOVER

Disease resistance in red clover (<u>Trifolium pratense</u> L.) generally is controlled by one to a few genes (Taylor and Smith, 1979). Inheritance of resistance to most red clover diseases is conditioned by dominant genes. The inheritance pattern and the number of genes involved for several diseases are summarized in Table 2.

For northern anthracnose, two (Sakuma et al., 1973) or more than three (Smith and Maxwell, 1973) dominant genes are involved. According to Hanson (1966) and Stavely and Hanson (1967), resistance to powdery

TABLE 1.	Causal	agents	and	distribution	of	red	clover	diseases.
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DISEASES	CAUSAL AGENTS	DISTRI- BUTION
BACTERIAL DISEASES: Bacterial leaf spot	<u>Pseudomonas syncingae</u> v. Hall	2,7
FUNGAL DISEASES: Blackpath Botrytis blight Cercospora leaf and	Rhizoctonia leguminicola Gough & Elliott Botrytis cinerea	12
stem spot Crown wart Fusarium root rot Myrothecium leaf		1,7 2,3,7 10
spot Northern anthracnose	<u>Myrothecium roridum</u> Tode ex Fries <u>M. verrucaria</u> Ditmar <u>Kabatiella caulivora</u> (Kirch.) Karak	
, Powdery mildew Pseudopeziza leaf sp Pseudoplea leaf spot Pythium blight	ot <u>Pseudopeziza trifolii</u> (BevBern.) Fck! <u>Pseudoplea trifolii</u> (Rostr.) Petr.	11 1. 10 11 10
Rust Sclerotinia root	<u>Uromyces trifolii</u> (Hedw. f. ex DC) Lev. var <u>fallens</u> (Desm.) Arth.	10
Sooty blotch	Sclerotinia trifoliorum Eriks.  Cymadothea trifolii (Pers. ex Fr.) Wolf  Collelitrichum destructivum O'Gava  Phoma trifolii E.M. Johnson & Valleau	13
Stagnospora leaf spot Stemphylium leaf	Stagnospora meliloti (Lasch.) Petr.	10
spot VIRAL DISEASES:	Stemphylium sarcinaeforme (Cav.) Wiltshir	e 10
Bean yellow mosaic Pea common mosaic Red clover vein mosai	ic ,	4,5,7,13 1,7 1
NEMATODE DISEASES: Stem nematode	Ditylenchus dipsaci	

1.	America:	central and
		eastern regions

- 2. America: northern area
- 3. America: southern area
- 4. America: southern and northern areas
- 5. Asia
- 6. Canada
- 7. Europe

- 8. Europe: southern regions
- 9. Pennsylvania
- 10. Red clover growing areas: generally distributed
- 11. Red clover growing areas: temperate zones
- 12. United States: southern regions
- 13. Africa

Source: Nyvall, R.F. 1979

TABLE 2. Inheritance pattern and number of genes involved for different diseases of red clover (<u>Trifolium pratense</u> L.)

RED CLOVER DISEASES

NUMBER AND KIND OF GENES INVOLVED

FUNGAL DISEASES

Northern anthracnose Powdery mildew

Rust

Two or three dominant One or two dominant One dominant, linked

with a seedling lethality factor

Southern anthracnose

One recessive

VIRAL DISEASES

Bean yellow mosaic Red clover vein mosaic One dominant One dominant

NEMATODE DISEASE

Stem nematode

Two dominant

mildew is: (a) monogenic dominant for five races of the fungus; (b) controlled by two genes in two races; and (c) variable among red clover clones. A single dominant gene controls the inheritance of resistance to rust (Diachun and Henson, 1974 a, b). However, this source of resistance can not be used for cultivar development because it is linked with a seedling mortality factor (Engelke et al., 1977). Southern anthracnose is the only fungal disease known to be controlled by one recessive gene (Athow and Davis, 1958). Crown rot resistance, investigated by Vestad in 1960, is believed to be heritable. Autotetraploid cultivars were proven to be more resistant to crown rot than comparable diploid cultivars. The effect of induced tetraploidy differs by genotype, suggesting that dosage effects of genes for resistance may be important.

The resistance to virus diseases of red clover appears to be inheritable. Three types of resistance to bean yellow mosaic virus, each controlled by a different dominant gene were reported by Diachun and Henson (1974 a, b). These are: a necrotic local lesion (hypersensitive reaction), resistance to mottling and systemic necrosis, and resistance to general mottling, controlled by a gene that appears to be epistatic to the gene for hypersensitive reaction. Red clover vein mosaic virus is controlled by a single dominant gene, Rc (Khan et al., 1978).

Resistance to stem nematode (<u>Ditylenchus dipsaci</u>) was reported by Nordenskiold (1971) to be regulated by two dominant genes, one of the genes being closely linked to the S-locus (self-incompatibility).

# II.3 ECONOMIC IMPORTANCE OF FUSARIUM ROOT ROT

Kilpatrick et al. (1954b.) reported heavy first-year losses in red clover seedlings at Madison, Wisconsin. For the first five weeks after seeding, stand loss averaged 42 percent. From the sixth through the eighteenth weeks, losses averaged another 45 percent. Only 1.2 percent

of the plants were still alive a year after seeding.

Several factors contribute to stand deterioration, but root and crown diseases are now recognized as the primary cause involved in the forage legume fields of Northeastern America (Leath  $\underline{et}$   $\underline{al}$ ., 1971).

A complete review of studies done on legume stand performance was reported by Fulton and Hanson in 1960. The majority of the research made on this subject prior to this date showed that the <u>Fusarium</u> species occurred in closed association with rotted red clover roots whenever the crop was grown. The majority of the studies done since then support this finding.

A survey, done by Aubé and Deschênes (1967), revealed that the crown and root rot diseases were present in every Quebec field surveyed and that the fungi would attack the plants at any stage of their development. Species of <a href="Fusarium">Fusarium</a> predominated among the fungi isolated from crown and root rots. Similar results were reported by Willis (1965) in Prince Edward Island.

# II.4 THE PATHOGEN: FUSARIUM SPECIES

# II.4.1 The taxonomy of Fusarium spp.

Several taxonomic systems were devised by mycologists to differentiate <u>Fusarium</u> species and forms (Snyder and Hansen, 1954; Messiaen and Cassini, 1968; Booth, 1971; Tousson and Nelson, 1976). A wide range of variation occurs between these systems. For example, a species named from one key may be identified differently, and be known under a different name with another key. According to Nelson <u>et al</u>. (1981), the Snyder and Hansen system constitutes the best method of classification for the numerous <u>Fusarium</u> species and should be used by a researcher whose interests lie more in the phytopathology than in the

systematics of these fungi.

Eusarium belongs to the subdivision Deuteromycotina, form-class Deuteromycetes (the imperfect fungi). They typically produce well-developed, septate, and branched hyphae with their compartments being usually multinucleated. Species of <a href="Eusarium">Fusarium</a> are differentiated mainly on the basis of the shape of their macroconidia. The basal cell of these fusiform spores may possess a distinct hook or notch depending on the species. The presence of microconidia and their shape and the presence of chlamydospores are other characteristics which differ between Fusarium species.

According to Toussoun and Nelson (1976), colony morphology, pigmentation, and other variable characters can be useful in the determination of isolates only after considerable familiarity with the genus has been acquired. The growing conditions should then be clearly specified such as light and temperature conditions, and the cultures should be started from a single spore grown on a defined medium.

The majority of <u>Fusaria</u> isolated from nature produce their macroconidia on sporodochia. That sporodochial type often mutates in culture as well as in nature. The mutant fungi in turn may give rise to other mutants, so that a mutational sequence is developed. In pathogenic isolates, these mutants frequently exhibit a loss in virulence. The mutation sequence has never been experimentally shown to reverse itself. Starting from the sporodochial type, mutations in general proceed in two opposite directions: (i) towards forms producing abundant aerial mycelium but few macroconidia, named mycelial types, and (ii) towards forms producing little or no aerial mycelium but abundant macroconidia, named pionnotal types. The mutants of mycelial types have a white, featureless look, while those of the pionnotal type have a shiny, wet

appearance.

Several factors will affect the sporulation of <u>Fusarium</u>. For most <u>Fusarium</u> spp., fluctuating temperatures ranging from 18' to 24'C are optimum. Light is essential for the production of macroconidia. Twelve hours of diffuse daylight from a north-facing window is usually sufficient. The optimum pH ranges from 5.5 to 6.5. Specific media, such as the Bilai and the Nash media (Tuite, 1969), are known to promote macroconidial production in <u>Fusaria</u>. According to the Snyder and Hansen system, <u>Fusarium roseum</u> is a conidial species, without microconidia. It grows rapidly, more than 0.4 cm per day at 25' and 18'C. The macroconidia are foot-celled.

# II.4.2 The occurence of Fusarium fungi with root rot diseases

In 1954, Kilpatrick et al. (b) attributed sixty nine per cent (69%) of all root and crown rot diseases occurring in Wisconsin red clover fields to Fusarium species.

Leath et al. (1971) reported the results of a survey of <u>Fusarium</u> occurrence in red clover fields covering the north-central part of the United States. A list of the pathogenic fungi found to be associated with the two-year old plants is given here in order of decreasing importance: <u>Fusarium oxysporum</u>, <u>F. roseum</u>, <u>F. solani</u>, <u>Trichoderma viride</u>
Pers. ex. Fr., <u>Gliocladium roseum</u> (Link) Thom, <u>Rhizoctonia</u> spp., <u>Phoma</u> spp., and <u>F. moniliforme</u>.

<u>Fusarium</u> species are common inhabitants of our soils. They are associated with several other plants. Gordon (1959) reported that approximately 9000 isolates of <u>Fusarium</u> were isolated from 173 plant species grown in Canada. The plants belonged to different families of commonly cultivated crops. Among the species isolated on Quebec red

clover, he named <u>Fusarium oxysporum</u>, <u>F. poae</u>, <u>F. acuminatum</u>, <u>F. solani</u> and <u>F. avenaceum</u>.

Willis (1965), working in Prince Edward Island, studied the extent and the incidence of root rot found on diseased red clover plants for the period ranging from three to seventeen months after seeding.

<u>Fusarium</u> species were the fungi most commonly isolated, followed by <u>Cylindrocarpon</u> spp., <u>Phoma</u> spp., and <u>Rhizoctonia</u> spp..

Aube and Deschenes (1967) studied the relative prevalence of fungi associated with crown and root rot of alfalfa and red clover at La Pocatiere and found that <a href="Eusarium">Fusarium</a> spp. were the most frequently isolated fungi.

The occurrence of <u>Fusarium</u> species was generally recognized around the sixties to be associated with <u>Fusarium</u> root rot in red clover. The question that remains to be answered relates to its pathogenicity. Several researchers argued that <u>Fusarium</u> species are weak pathogens that invade the roots only if the plant was already infected by another pathogenic fungus or weakened by adverse environmental conditions. This lead to further research which tried to elucidate this question.

# II.4.3 The pathogenicity of Fusarium species on red clover roots

Isolates of <u>Fusarium</u> species vary in their capacity to induce root rot in forage species. Kilpatrick, Hanson and Dickson (1954a) suggested that isolated <u>Fusarium</u> were specific in host range, i.e. that their virulence was restricted to a single host species or to closely related species.

Fulton and Hanson (1960) studied the pathogenicity of 40 fungus isolates coming from naturally infested red clover roots under laboratory conditions in Wisconsin. They established that isolates were generally more pathogenic on seedlings than on older plants and that a

wide range in pathogenicity occurred between isolates. The fungi tested are reported here in order of decreasing pathogenicity: <u>F. oxysporum</u>, <u>F. solani</u>, <u>F. roseum</u>, <u>F. moniliforme</u>, <u>Gliocladium roseum</u> and <u>Rhizoctonia</u> spp..

Chi (1965) tested 36 isolates of <u>Fusarium</u> on seedlings of Lakeland red clover. Tested under laboratory conditions, the isolates differed greatly in virulence. These isolates, coming from naturally infested Canadian red clover, are enumerated in decreasing order of aggressiveness: <u>F solani</u>., <u>F. oxysporum</u>, and <u>F. roseum</u>. Among the species tested, most of those belonging to the Fabaceae family became diseased, while none of the species belonging to the Brassicaceae, Poaceae and Solanaceae showed any infection. Alternatively, all the <u>Fusarium</u> species isolated from cabbage, tomato and pea did not infect the clovers. Chi (1965) concluded that <u>Fusarium</u> isolates are specific in host range since their virulence is restricted to a single host species or species belonging to the same genus or family.

Leath and Kendall (1978) also showed that isolates of <u>Fusarium</u> are generally more virulent on the host species from which they were originally isolated than on any other species.

# II.5 INOCULATION TECHNIQUES USED TO INDUCE ROOT ROT

Inoculation techniques used to induce <u>Fusarium</u> root rot in forage legume crops have varied with the goals of the experiment. The two main approaches generally used were: a. histological studies of the fungi and/or b. selection of resistant genotypes to specific <u>Fusarium</u> species in a plant population. The methodology used for histological studies might be used to do some selection. But, in general, it is much more meticulous and time consuming than the methods developed for selection

# II.5.1 <u>Histological methods used mainly to test the pathogenicity</u> of Fusarium species

Testing the pathogenicity of various <u>Fusarium</u> isolates is often done under laboratory conditions. Chi <u>et al</u>. (1964) studied the penetration and subsequent development of three <u>Fusarium</u> species in alfalfa and red clover. They took a petri dish containing PDA and placed a piece of 5-day-old <u>Fusarium</u> culture at its center. They radially disposed 15 sterilized seeds around it at a distance of 2 cm. Three days after germination of the seeds, they made their miscroscopic observations.

Chi (1965) placed 3-day-old red clover seedlings on a glass wool platform placed at the bottom of a test tube. The latter contained 10 ml of Hoagland's solution. The seedlings were inoculated by placing six 5 mm discs of 7 day-old <u>Fusarium</u> inoculum, grown on PDA, on top of the wool platform. Disease assessment was recorded 10 days after inoculation.

The most popular inoculation method used for histological studies under greenhouse conditions is often referred to as the "bare-root-soak technique (BRST)" (Richard et al., 1980). It consists of dipping the bare-root of a plant, previously cultivated in a soiless medium, into a suspension of a known Fusarium concentration.

Chi (1965) dipped the bare roots of two-week-old red clover seedlings in a 10-day old <u>Fusarium</u> broth culture. He then transplanted them in 36 by 51 cm flats, each containing about 14 kg of a soil mixture or white silica sand. Five hundred ml of the spore suspension, containing approximately 100,000 cells per ml, were added to each flat immediately after transplanting. Disease assessment was done 65 days

after transplanting.

A more sophisticated method, called the "slant-board culture technique" was developed by Leath and Kendall (1974, 1978). The plant, grown on a cafeteria type of tray, is nourished with a hydroponic solution. The roots are well spread on the surface of the tray and are covered with an aluminium sheet. This technique allows a direct application of a known concentration of a fungus at a specific level on the root. It is a precise, space efficient technique, but requires a lot of skill to manipulate the hydroponic system.

# II.5.2 Mass screening technique against Fusarium root rot

The "application technique (AT)", devised by Leath and Kendall in 1978, is now widely used by plant breeders (Pederson  $\underline{e}\underline{t}$   $\underline{a}\underline{l}$ , 1980; Richard  $\underline{e}\underline{t}$   $\underline{a}\underline{l}$ , 1980). It is a very fast method, well suited for conventionnaly equipped experimental stations and does not require any special expertise to use.

The plants are cultivated in a sterilized soil media. The taproot of the legume is cut at a constant level below the crown and the <a href="Fusarium">Fusarium</a> mycelia directly applied to the wounded taproot. The plant is then repotted until disease assessment.

Pederson et al., (1980) applied a 5-day-old <u>Fusarium</u> culture, grown on PDA in the dark at 22°C, on six month old red clover roots at about 4 cm below the crown.

# II.6 SYMPTOMS ASSOCIATED WITH THE FUSARIUM ROOT ROT DISEASE

<u>Fusarium</u> root rot induces specific symptoms in red clover; however, any of the symptoms observed can be associated with any of the <u>Fusarium</u> species that colonize the host tissue. Red clover plants may be affected in all stages of their development, the first symptoms appearing on the

attacked plant parts, the roots, while the aerial plant parts remain healthy. In fact, the crowns or roots of clover plants are usually severely rotted before the tops even start to wilt and eventually die. In other cases, the plants are weakened through partial rotting of the crowns or tap roots (Martens et al., 1984).

Necrotic areas are often associated with wounds in the crown or root surface. They are frequently confined to the cortex of the taproot and lateral roots, but in some cases discoloration is restricted to the central core and may follow the vascular system. As the disease develops, both the cortex and central core may be invaded by the fungus.

When sufficient root tissue is killed, plant vigor declines (Leath et al., 1971). Plants then appear unthrifty, stunted, yellowish, and wilt during hot, dry summer days (Nyvall, 1979). If the invasion continues, the plant dies. Commonly, when the taproot decays the plant is maintained by new lateral roots which develop near the crown; however, these are often invaded and killed also (Leath et al., 1971).

# II.7 MEASUREMENT SCALES USED TO ASSESS FUSARIUM ROOT RDT

Disease intensity can be expressed differently depending on the type of measurement used. When diseased plants or plant parts are total losses, counts of diseased plants or plant parts and conversion of the counts into percentages gives an accurate measure of the disease intensity. Provided a diseased plant or an organ is properly defined, this method will be uniform from one worker to another.

In an effort to standardize disease estimation by researchers, James (1971) produced an illustrated series of assessment keys for plant diseases. However, when different plants or organs differ appreciably in their amount of disease, or when, for any other reason, the amount of damage is not correlated with the percent of diseased plants or organs.

this method does not appear to be appropriate. The root rot diseases belong to the latter category. In such cases, a combination numerical method is often used or the number of plants or organs is recorded in each of several disease percentage classes, and reduced to a single expression of disease intensity, such as the Horsfall-Barratt scale.

# II.7.1 The Horsfall-Barratt scale

The Horsfall-Barratt scale was developed by Horsfall and Barratt in 1945. This logarithmic scale estimates the percentage of plant disease, based on the Weber-Fechner law which states that visual acuity is proportional to the logarithm of the density of the stimulus.

The scale is divided from grade 0 to 11, the difference between each grade being large enough to be distinguishable by eye. In this scale, the units pass through 1.5 logarithmic phases in each direction from the 50% point, on which equal linear distances are called equal probability or "probits" (Chester, 1950). This was based on the assumption that up to 50%, the eye tends to judge the total area of plant tissue that is diseased, while above 50%, the eye judges the percentage that is healthy. The relationships between the grades and the corresponding % diseased and % healthy areas are given in Table 3.

But, as the Horsfall-Barratt system is increasing in popularity, some researchers are seriously questioning its validity. Hebert (1982) argues that the initial hypothesis on which this grading scale is based is false: not all estimates relying upon visual perception obey the Weber-Fechner law as many factors may affect the stimulus-response curve besides the sensitivity of the observer to the stimulus.

TABLE 3. Relationships between the rating scores of the Horsfall-Barratt scale and the estimated mean percentages of diseased and healthy tissues.

Horsfall-Barratt

GRADE #	DISEASED %	HEALTHY %	GRADE FORMULA %
0	0	100	1.17
1	0-3	97-100	2.34
2	3-6	94-97	4.68
3	6-12	88-94	9.37
4	12-25	75-88	18.75
5	25-50	50-75	37.50
6	50-75	25-50	62.50
7	75-88	12-25	81.25
8	88-94	6-12	90.63
9	94-97	3-6	95.31
10	97-100	, 0-3	97.66
11	100	0	98.82

Source: Redman, King, and Brown of Eli Lilly Company (Elanco Division)

#### II.7.2 Other numerical scales

In a field survey of root and crown rots of red clover in Wisconsin, Kilpatrick et al. (1954b) segregated the diseased plants into seven classes, according to the amount of root decay. These classes and the values assigned were as following: healthy=0, trace=5%, slight=20%, moderate=40%, moderately severe=60%, severe=80%, and very severe=95%. After classification, the number of plants in each class was multiplied by the class value, the sum of the products determined and the latter divided by the total number of plants in each classes to give the average disease severity rating for the sample.

Fulton and Hanson (1960) evaluated the occurence of root rot in Wisconsin using disease indices based on nine severity classes, ranging from 0 to B. Zero indicated no root discoloration, while eight was given to roots very severely rotted, the plants being nearly dead. Intermediate levels of disease were distributed between the 2 and 7 scale.

Willis (1965) studied the incidence of root rot in Prince Edward Island. He used a disease severity index where plants were assigned to one of the following five classes: healthy=0, trace=1, slight=2, moderate=3, severe=4. After classification, an average disease rating was calculated for the sample. The same scale was used by Gagnon (1979) with red clover and by Richard et al. (1980) with alfalfa.

Other researchers used a similar disease rating scale, but ranging from 0 to 5. Chi and Hanson (1961) and Chi (1965) used it as they were working on the pathogenicity of red clover seedlings. Viands et al. (1979), Viands and Barnes (1980) and Richard et al. (1982) used it to rate <u>Fusarium</u> wilting in alfafa. They based the disease severity index on surviving plants only. The classes were defined as following: 0= no

disease or root discoloration; 1= trace of root browning or an occasional lesion, mostly on secondary roots; 2= slight to moderate root browning, considerable necrosis on secondary roots; 3= moderately severe rotting of secondary roots and tap roots; 4= severe rotting of entire root system; 5= very severe rotting; plant killed.

Leath and Kendall (1978) introduced the idea of measuring the length of vertical discoloration from the inoculation site towards the crown with the introduction of the slant-board culture technique. They later developed another inoculation technique, described as the "application technique", which would allow measuring the penetration of the fungi the same way. Measurement of the vertical discoloration from the inoculation point was used by Pederson et al. (1980) with red clover, and by Richard et al. (1980) with alfalfa. These latter researchers decided to use a combined index in some part of their experiment, multiplying the length of vertical discoloration by a rating score ranging from 0 to 5, in order to obtain an estimation of the volume occupied by the disease within the root.

# II.8 FACTORS INFLUENCING SUSCEPTIBILITY OF RED CLOVER TO FUSARIUM ROOT ROT

# II.8.1 <u>Varietal differences</u>

Variations in susceptibility to <u>Fusarium</u> root rot disease among cultivars has led some workers to believe that there is a possibility of genetic improvement. Several results reported by different research groups confirmed this possibility. As early as 1950, Kilpatrick and Hanson reported on an experiment where losses due to root rot varied from 39% in the cultivar Dollard to 52% in the cultivar Emerson, with an average stand mortality of 45% over all cultivars tested. A year later, Crall (1951) working on a wilt disease of red clover seedlings in Iowa,

reported that, in a greenhouse experiment one isolate of <u>F. oxysporum</u>, after 3 months of incubation, caused the following stand reductions: Emerson 33%, Midland 30%, Kenland 18% and "common red clover" 36%. Kenland was most resistant and "common" most susceptible to all root rot pathogenic isolates.

At two locations in the province of Québec, Gagnon (1979) observed the evolution of root rot development on several red clover cultivars. He reported that the cultivar Hungarapoli was the most susceptible to <a href="Fusarium">Fusarium</a> root rot followed by the cultivar Ottawa. Cultivars Dollard and Lakeland showed equivalent reactions and were the least affected by root rot.

# II.8.2 Changes in host susceptibility with time

Plant susceptibility to disease varies with plant age and also responds to the influence of changing environmental factors. Changes in susceptibility with age and time of year are the most important factors (Horsfall and Cowling, 1978). In perennials, the effects of the yearly environmental cycle on susceptibility are closely associated with the effects of the age of the herbaceous shoots and new vascular increments. However, the susceptibility may also change over the years after successive generations of annual shoots and annual layers, and thus plant age becomes a factor clearly dissociated from the yearly environmental cycle. Willis (1965), working in Prince Edward Island (Canada), stated that the relative prevalence of <u>Fusarium</u> spp. was higher on first-year plants than on second-year plants, and was the highest in the youngest (3-month old) seedlings. <u>Cylindrocarpon</u> spp. were, however, more commonly isolated from the oldest plants studied. Aube and Deschenes (1967) isolated <u>Fusarium</u> spp. more frequently from 1-

year old plants than 2- or 3-year old plants. Bagnon (1979) observed different patterns of infection at the La Pocatiere and Normandin Research Stations, which he attributed to the differing snow cover and winter severity between these two locations.

# II.8.3 Relationship of clipping to root and crown deterioration

Several reports conclusively demonstrated that clipping the foliage of red clover increased susceptibility of the plants to root rots. Fulton and Hanson (1960) stated that this was true regardless of whether the plants were grown in sand or fine textured soil, and regardless of the age of the plants at the time of clipping. The magnitude of increase in susceptibility is influenced by the frequency of clipping, the age and vigor of the plants when clipping is done, as well as the temperature, the presence of pathogens and other factors.

#### II.9 THE GENETICS OF FUSARIUM ROOT ROT IN RED CLOVER

Breeding for resistance to <u>Fusarium</u> root rot in red clover might appear somewhat unrealistic because of the complexity of the problem itself. Tetteh (1980) reported the reasons enumerated by Dijkstra in 1964:

- a. lack of adequate knowledge about the nature of resistance to clover rot
- b. absence of a high degree of resistance
- c. dependence of the resistance on the vigour of the plant
- d. dependence of the aggressiveness of the fungus on environmental conditions

Leath  $\underline{e}\underline{t}$   $\underline{a}\underline{l}$ . (1971) suggested that breeders should rather concentrate their efforts towards developing varieties more tolerant to the stress factors commonly occurring in a particular area.

Working with progenies of diallel crosses in Arlington red clover,

Pederson et al. (1980) attributed <u>Fusarium</u> root rot resistance to genes with additive effects. Obtaining a very large error variance for their experiment, they suggested that mass or phenotypic recurrent selection would probably not be effective in breeding for root rot resistance. They recommended the use of progeny tests for family or modified earto-row selection because these methods would provide some control over the environmental variance.

As a test of this recommendation, Smith (1983), using the phenotypic recurrent selection scheme for breeding resistance to several diseases in red clover, did not find any decrease in the incidence of <u>Fusarium</u> root rot diseases after a first cycle of selection. However, he obtained excellent results while selecting against other aerial fungal diseases such as northern anthracnose, powdery mildew, leaf rust and target spot.

#### II.10 SEXUAL REPRODUCTION OF RED CLOVER

# II.10.1 Floral structure

Red clover belongs to the Fabaceae family, subfamily Papilionoideae, tribe Trifoliaeae. Its inflorescence is a head containing 100 to 200 flowers. Each flower measures from 13 to 20 mm, and is typically zygomorphic, the corolla being of papilionaceous configuration (Gleason and Cronquist, 1963). The perianth of each flower is 5-merous. The calyx, glabrous to sparsely pilose, is made of five (5) united sepals about 2 to 5 mm long, the upper one being about 2 mm longer than the other petals. The corolla is formed by five (5) unequal petals, namely a standard (the uppermost petal), two lateral wing petals, and two basal fused petals (the keel). The androecium consist of ten (10) stamens in a diadelphous arrangement. A superior overy surmounted by a unique style

and a stigma, constitutes the gynoecium. The androecium and the gynoecium lie between the keel petals. The color of the corolla varies from magenta to nearly white.

# II.10.2 Natural pollination

The best natural pollinators appear to be the bumblebees (Bombus spp.). However, the honeybees (Apis mellifera L.) are often used even if they might avoid the clovers if other sources of pollen and nectar are available. Other bee species such as the alkali bees (Nomia melanderi Ckll.) and the leaf cutter bee (Megachile rotundata (F.)) are also used for red clover pollination. Usually, when a red clover flower is pollinated by a bee, its sexual column protrudes from the interior of the flower, with its pistil extending slightly beyond the stamens. When the weight of the bee is removed, the sexual column returns to its original position (making red clover a non-tripping species) (Taylor and Smith, 1979).

# II.10.3 Artificial crossing

In order to make specific crosses, precautions must be taken to avoid the presence of pollinating insects among the flowering plants. In the field, it is necessary to protect the heads of each plant from accidental pollination prior to flowering by covering them with bags of fine muslin about 9 X 14 cm, which can be closed with a draw string (Taylor, 1980). In the greenhouse, simply avoid introducing any insect within the nursery. Windows must be covered with a fine net and doors must remain closed.

Manipulation of the red clover flower heads requires some precaution in order to reduce the breakage of the stems. Wires or stakes, appropriate for the height of the plants, are placed around the plant to

support the bagged heads. Prior to crossing, the flower heads are trimmed to keep only the ones which present the proper stage for optimum seed set. The optimum stage of flowering to pollinate red clover is when the flowers are about half opened. To ease manipulations, the heads are trimmed to 15 to 20 newly opened flowers in the center of the head (Taylor, 1980).

Because of the self-incompatibility system in red clover, emasculation is usually not necessary. This self-incompatibility is controlled by the gametophytic S-allele system. However, there are some self-fertile stocks of red clover, and in these cases, emasculation is desirable. Emasculation of self-pollinated species is quite difficult because the flowers may be small and tightly packed in the head. Furthermore, the anthers may dehydrate at a very early stage, sometimes before the petals are extruded beyond the calyx.

#### II.10.4 Pollination

The first heads to bloom in red clover are located at the top of the main stems. Red clover flowers first open in the middle of and on the topmost part of the heads. In red clover, the highest seed set when cross-pollinating is obtained with flowers about half opened (Taylor, 1980). Stigma receptivity and pollen viability continue after this stage for about ten days, but gradually decline under greenhouse and field conditions (Taylor and Smith, 1979).

In manual pollination, the pollen is removed from the donor plant by inserting the pollinating instrument (ex. toothpick) between the standard and the keel. A downward pressure applied on the latter causes the staminal column to strike the toothpick. Pollen quality should be checked at that time. Viable pollen looks moist and is yellow, while dead pollen appears dried and whitish.

Pollen is transferred by the pollinating instrument to the stigmas of plants designated as female parents. For reciprocal crosses, pollen is collected and applied alternately between paired heads of different plants using the same toothpick. One collection of pollen will usually pollinate 10 to 15 emasculated flowers. However, pollen from unemasculated female plants will tend to dilute that of the donor male plant, and usually only 5 to 10 flowers are effectively cross-pollinated. After all flowers of a particular cross have been pollinated, a small tag is looped and secured on the stem immediately below the head, and is labeled as to the parentage and the crossing dates. Heads are kept free of water for at least the first 24 hours to prevent abortion of pollen.

Before proceeding to the next cross, hands, forceps and other pollinating equipment are washed with alcohol and rinsed with water. If pollinating instruments are to be reused, they are set aside for several days after washing to prevent contamination (Taylor, 1980).

# II.10.5 Seed development, harvest and storage

It takes 28 to 35 hours between pollination and fertilization of the egg cell in diploid red clover. Each ovary contains two ovules, but except for some strains, one usually aborts (Taylor and Smith, 1979). However, up to four ovules per ovary have been found (Povilaitis and Boyes, 1959). Pollinated red clover flowers usually begin to wilt in about 2 days, while non-pollinated ones remain unwilted up to 10 days after blooming. The seeds are physiologically mature 14 days after pollination, and are dry enough for harvest after about 21 days. The ripening process may be delayed by humid conditions (Taylor, 1980).

#### II.11 THE CULTIVARS ARLINGTON AND FLOREX

Two red clover cultivars were chosen because of their good potential for Northeastern Canadian forage production. Their characteristics and breeding history are described below.

#### II.11.1 Arlington

Released in 1973 by the United States Department of Agriculture in cooperation with the Research Division of the College of Agriculture and Life Science of the University of Wisconsin-Madison, Arlington is a double-cut red clover intended for use in forage production in the north Central United States. This cultivar is registered with the Crop Science Society of America (Reg No. 16, Crop Sci. 13:771, 1973). It obtained its Canadian license (No. 1888) in 1979 from Agriculture Canada. The breeding procedures used to develop Arlington are reported by Smith et A polycross progeny of plants selected for field al., persistence from the cultivars Chesapeake, Dollard, Kenland, Pennscott, Rahn, Van Allen and Wisconsin Mildew Resistant served to make up the six initial populations to start the breeding program. Three cycles of selection were applied to each of the six heterogeneous populations for persistence and resistance to northern anthracnose, powdery mildew and bean yellow mosaic virus. After the third cycle of selection, 30 selected plants from each population were intercrossed and seed was harvested in bulk. About 1,000 plants from the bulked seed were screened a fourth time for resistance to the above mentioned diseases, and 300 selected plants were intercrossed and the seeds harvested in bulk.

In the American registration (1973), Arlington is said to be resistant to powdery mildew (causal agent: <a href="Erysighe golygoni DC">Erysighe golygoni DC</a>.) and northern anthracnose (causal agent: <a href="Kabatiella cauliyora">Kabatiella cauliyora</a> (Kirchn.) Karak), with moderate resistance to bean yellow mosaic virus. Trials

conducted in Ontario (Canada), confirmed that it was tolerant to these diseases.

Arlington yielded significantly more forage, over a 3-year period at Madison (Wis.) than Lakeland, Dollard and Common (Wis.) red clover cultivars. Arlington's yield was estimated 13% higher than that of Lakeland. In Canada, the Ontario Forage Crops Committee concluded that it was well suited for our prevailing climatic conditions, and it is listed among the recommanded cultivars of Quebec and Ontario.

## II.11.2 Florex

Florex is a double-cut red clover developed by a private American company, the Northrup King Co. (Minneapolis, Minnesota). Released in the United States in 1976, it obtained its Canadian license (No. 1789) in 1977. Agriculture Canada (1978) described its breeding scheme as follows:

"The source material came from a Dollard population, established at the Northrup King Research Center, Eden Prairie in 1959. Remnant plants were removed in the spring of 1965, the crowns split, and only those free of crown breakdown saved. These were recombined in isolation. A new broadcast seeding was established in 1966. In 1970, persistent clones were again dug and evaluated for healthy crowns and vigourous growth. Two hundred plus plants were recombined in isolation to produce breeder's seed. During the two cycles, selection was made for resistance to powdery mildew, northern anthracnose, and rust."

In U.S. trials, approximately 65% of the plants have exhibited some resistance to powdery mildew. Under Minnesota and Iowa field conditions.

where observation plantings have been observed through four hay years, it was noted that Florex was substantially more persistent than Dollard. In the Ontario Forage Crops Committee trials (Canada), Florex was tested and is now recommanded as a suitable cultivar for Quebec and Ontario.

#### III. MATERIALS AND METHODS

#### III.1 BIOLOGICAL MATERIAL USED

#### III.1.1 Red\_clover\_plants

About 300 certified seeds of each red clover cultivar, Arlington and Florex, were obtained from the E. A. Lods Agronomy Research Centre of Macdonald College of McGill University. These seeds had been kept for one year in cold storage.

## III.1.2 <u>Fusarium isolates</u>

Three <u>Fusarium</u> isolates (#B14, 927 and 959) were received by mail in October 1981 on PDA slant cultures through the generosity of Dr. K. T. Leath of the United States Regional Pasture Research Laboratory, Pennsylvania. Information on the isolates, provided by Dr. Leath, and reported by Tetteh (1980) is presented in Table 4.

In order to present a physical description of each Fusarium isolate for future reference, the fungi were grown on PDA (to observe mycelial characteristics) and on Bilai medium (for macroconidial The PDA medium was prepared by dissolving 11.5 g characteristics). of PDA in 250 ml of distilled water in a 500 ml Erlenmeyer flask. flask was sealed with non-absorbent cotton and autoclaved at 103 kilopascals at 121'C for 13 minutes (Pelletier, 1981). modified by Joffe (1963), is known to induce conidial medium, development in Fusaria.

TABLE 4. Information on <u>Fusarium</u> isolates #814, #927 and #959.

FUNGUS SPECIES	ISOLATE NUMBER	ORIGINAL HOST	SOURCE	PATHOGENICITY
Eusarium roseum	814	Red clover	Pennsylvania	Not determined
F. roseum "Acuminatum"	927	Alfalfa	Pennsylvania	Pathogenic on Alfalfa and Red clover
E. roseum "Acuminatum"	959	Red clover	W. Virginia	Alfalfa and Red clover

The solidifying agent, 10 g of Bacto-agar (0.6%), was added to the following ingredients:

INGREDIENTS	GRAMS
Potassium phosphate monobasic (KH2PO4) Potassium nitrate (KNO3) Magnesium sulfate (MgSO4) Potassium chloride (KC1) Starch powder Glucose Sucrose Water	1.0 1.0 0.5 0.5 0.2 0.2 0.2

The original pH of the solution (5.2) was brought up to 5.9 with the addition of NaOH (0.1N). Photographs were taken of cultures grown on PDA medium left in a dark incubator set at 21°C for seven days (Figure 1). Photograph of a macroconidia produced by isolate 814, after seven days of growth on Bilai medium, was also taken (Figure 2).

#### III.2 CULTURE OF RED CLOVER PLANTS

#### III.2.1 Seeding and maintenance of parental material

The Florex and Arlington parental plants were sown on June 4 1981 in a greenhouse bed. The bed, of 20 cm depth, was filled with a soil medium of pasteurized soil, peat moss and vermiculite (2:1:1). Prior to seeding, the clover seeds were inoculated with a viable powder of Rhizobium trifolii. The seedlings were thinned, by June 25 1981, to keep 220 plants per cultivar, in rows about 4 cm apart.

The greenhouse bed was fertilized immediately after seeding with 5-20-20 at the rate of 6 g/liter of water. Thereafter, and once every month, they were alternatively fertilized with 0-15-30 at the rate of 4 g/liter of water, or with 5-20-20 at the rate of 6 g/liter of water. Plants were watered with tapwater. This parental material remained in the greenhouse bed through the winter of 1981-82, under natural light conditions, while the temperature was maintained around 18°C. Their

FIGURE 1. Mycelium characteristics of the three <u>Fusarium roseum</u> isolates (814, 927 and 959) grown under identical environmental conditions.

FIGURE 2. Macroconidiae of Fusarium roseum.





foliage was cut off every 40 to 50 days during the growing period (March to October) while during the rest of the year (November to February) no cutting was required.

Plants were transplanted from the beds to individual 5-inch pots by May 16, 1982. The potting mixture was made of soil-promix (1:1). Immediately after transplanting, the plants were fertilized with 10-52-10 at the rate of 10 g/liter of water. Thereafter, they were fertilized every month with 0-15-30 at the rate of 4 g/liter of water. These parents were transplanted again into individual 6 inch pots by June 1983. Their roots and foliage were then severely trimmed to allow new growth and regeneration.

## III.2.2 Reproduction and maintenance of red clover genotypes

III.2.2.1 VEGETATIVE REPRODUCTION. In order to obtain replicates of each genotype present in the initial parental population while keeping the original plants alive for further investigations, stem cuttings of the parental materials were taken on 2-month old plants on August 30 These cuttings were axillary branches at the crown level of the mother plant. As many tillers as possible were taken from each genotype (the number varied from one to three, depending on the vigor of the mother plant). Each genotype was identified by a different number, and their propagules by the number of the mother followed by a decimal (example: plant 131.2 indicates the second propagule of mother #131). These cuttings were dipped in an indole butyric acid rooting powder for softwood (Seradix #1, May & Baker Ltd.). Then, the cuttings were put in an intermittent mist frame for a period of approximately three weeks. depending on the root development of the cuttings. The medium in the mist frame was composed of 1:1 peat-moss and perlite. It had previously been used for plant vegetative propagation of several horticultural

species. Three soil samples were taken diagonally through the mist and cultured on PDA petri dish to determine if any <u>Fusaria</u> were present in this medium. No traces of <u>Fusaria</u> were found.

III.2.2.2 Maintenance. The rooted propagules were then planted in 6 inch plastic pots, 4 plants/pot. This operation was done on September 24th, 1981, as the plants were one month old. The soil mixture used was 2:1:1 sterilized soil, peat moss and vermiculite. The soil was sterilized to ensure the action of <a href="Eusarium">Fusarium</a> isolates that would be inoculated later. A container filled with about 70 kg of soil was autoclaved at 103 kPa at 121°C for 8 hours. Cuttings were fertilized immediately after transplanting with 10-52-10 at the rate of 10 g/liter of water.

Two weeks after transplanting (October 8th, 1981), the young plants were watered with a suspension of <u>Rhizobium trifolii</u> in order to induce nodulation. From then, fertilization occured every two-to-three weeks with 5-20-20 at the rate of 4 g/liter of water. The fertilization program was stopped two weeks before inoculation of <u>Fusarium</u> started.

The plants were allowed to grow until December 17th, 1981, in this situation. As they were four months old, some plants were checked for taproot development and the possibility of starting the inoculation of <a href="Eusarium">Eusarium</a> isolates. At that time, the plant had not developed a large enough taproot, and the secondary roots of all four clones were so inter-mixed that it was difficult to distinguish which roots belonged to each propagule. The plants were therefore transplanted to individual 5-inch standard pots. Another application of 10-52-10 (at 10 g/l water) was given to the plants. Thereafter, the same fertilization scheme, as described above, was applied until two weeks before inoculating with the <a href="Eusarium">Eusarium</a> isolates.

## III.2.3 Seeding and maintenance of F1 progeny

To study the inheritance of <u>Fusarium</u> root rot, a number of crosses were made among resistant and susceptible plants. A group of F1 progeny was seeded October 16, 1983. The seeds had been stored in the cold for two months, and then scarified two minutes with sand paper (180 mesh) prior to planting. Seeds were sown in flats containing individual plastic tubes filled with a fine mixture of sterilized soil, peat-moss and vermiculite (1:2:2). The seeds were covered with a viable powder of <u>Rhizobium trifolii</u> prior to seeding. The flats were fertilized with 5-20-20 at the rate of 4 g/l of water every two weeks until they were one month old. Progeny plants were under artificial sodium lights. These lights were placed at 3 feet above the growing flats and the daylength was set at 14 hours to promote vegetative growth (Cumming, 1956).

Seedlings were transplanted into individual 4 inch pots when they reached six weeks old, by November 27th, 1983. One application of 10-52-10 at 10g/l of water was applied to the transplants. Thereafter, one cup per plant of a 5-11-26 plus micronutrients solution (10 g/ml water) was given every two weeks. Plants were cut back to 8 cm above the soil surface two weeks before inoculation begun.

#### III.3 CULTURE OF FUSARIUM

# III.3.1 Long-term preservation on soil medium

Two pure cultures of each fungus were stored in a soil medium in 20 ml vials which were kept in a refrigerator maintained at 4'C. A soil medium in the vials was selected because the virulence of fungi had been reported to decrease when kept on artificial media such as PDA for a long period (Tousson and Nelson, 1976). This soil medium was composed of 10 g of finely screened soil (0.5 mesh), of 0.5 g corn meal and of 5 ml

of distilled water. The vials were covered with aluminium foil in order to prevent light penetration within the vials, which might induce mutation in the fungal culture (Tuite, 1969). Before inoculation, the caps were gently tightened and the vials were autoclaved at 103 kPa and 121°C for three minute periods with intervals of 24 hours between them (Tuite, 1969). Two days later, <u>Fusarium</u> cultures were established by transferring pieces of the original cultures to the vials using aseptic techniques. These cultures were sealed and set aside at room temperature for two more days, after which they were stored in the refrigerator for future use.

As a precautionary measure, six PDA slant cultures were prepared and kept in the refrigerator at 4°C. These were transferred to new PDA slant cultures every two months. Test tubes (18 X 150 mm), filled with 15 ml. of PDA, were autoclaved 13 minutes at 103 kPa at 121°C (Tuite, 1969).

# III.3.2 <u>Inoculum preparation</u>

For each soil culture series derived from the <u>Fusarium</u> cultures, a vial was removed from the refrigerator and was allowed to remain at room temperature for one day. Meanwhile, sterile petri dishes were filled with 15 ml of PDA, and, as they were cooling, seven strips of sterilized polyester cloth (1 cm X 2 cm) were radially distributed on the surface of each dish. The polyester strips were sterilized by submerging in distilled water in a glass petri dish and autoclaving at 103 kPa at 121°C for two 30 minutes periods with an interval of 24 hours between each sterilizing period. Once the PDA was completely cooled, a hyphal tip of the fungus isolate from the appropriate vial was introduced into the center of each petri dish under aseptic conditions. The dishes were then sealed with laboratory parafilm and placed in the dark in an incubator set at 21°C for seven days.

## III.4 SCREENING RED CLOVER PLANTS FOR FUSARIUM RESISTANCE

#### III.4.1 Application of Fusarium to red clover roots

The application technique (AT), as described by Leath and Kendall (1978) and by Richard <u>et al</u> (1980), was used to inoculate the fungus on red clover plants. The procedure, illustrated in Figure 3, is described below:

- 1. The soil root mass is removed from the pot;
- The roots are cut transversely through the sod, about 3 cm below the crown;
- An inoculum strip is placed against the cut end of the taproot;
- 4. The root mass is reassembled and returned to the pot.

The inoculated plants were allowed to grow for four (4) weeks. Special care was given to keep the soil uniformly wet during that period.

#### III.4.2 Evaluation of the degree of root rot

The plants were dug out and the soil was washed from the taproot with tapwater. The whole plant was then put into a numbered paper bag. Not later than 3 hours after removal from the pot, plants were divided into two parts: the aerial foliage and the underground roots, The point at which the plant was bisected was determined by estimating the middle of the shoot. The foliage was set aside while the roots were evaluated for root rot.

Each root was immersed in alcohol (70%) for 10 sec, then transferred to a chlorine solution (3.5% sodium hypochlorite) for 2 minutes, then rinsed two consecutive times with sterile water. Each solution was changed after every tenth root because of the accumulation of soil particles. Roots were split longitudinally under aseptic conditions.

FIGURE 3. The application technique (AT) used to inoculate the red clover taproot with <u>Fusarium</u>.

FIGURE 4. <u>Fusarium</u> root rot assessment in a red clover taproot inoculated using the application technique.





Infected tissues of control plants were isolated and transferred to petri plates, containing 15 ml of PDA with an antibacterial solution, before further manipulations of the taproot. The antibacterial solution was made of chlorotetracycline HCl (0.4 mg/ml of water) and streptomycin sulfate (1.5 mg/ml of water). Ten ml of this solution, filtered through a millipore filter (pore size: 0.2 um), was added to each 100 ml of sterilized PDA. Random reisolation of <u>Fusarium</u> from infected tissue of treated plants was performed using the same medium as for control plants, in order to conform with Koch's postulates.

The incidence of Fusarium root rot disease (Figure 4) was evaluated by two different methods. The first method involved measuring the length of the vertical discoloration observed from the wounded site of the split root. The second method consisted of the evaluation of the proportion of healthy or diseased tissue which remained in the diseased roots (expressed in percentage of the total area). The latter is referred to as the Horsfall-Barratt grading system, set up by the research laboratory of the Eli Lilly Company, U.S.A. (Horsfall and Barratt, 1945). Pictures of alfalfa root rot graded according to this scale are illustrated in Figure 5 (Courtesy of C. Richard, Agriculture Canada Research Station, Ste-Foy, Que.). Two persons evaluated the disease incidence simultaneously. First, each individual determined independently a Horsfall-Barratt scale. The mean of both results was taken as the correct number. After this, one of the persons wrote the results down while the other was measuring the length of the vertical discoloration and taking complementary data.

The whole plant was dried in a paper bag placed in a forced-air oven at 82.5°C for 24 hours. Weighing was done with an electronic balance.

FIGURE 5. Representation of the Horsfall-Barratt scale in alfalfa.

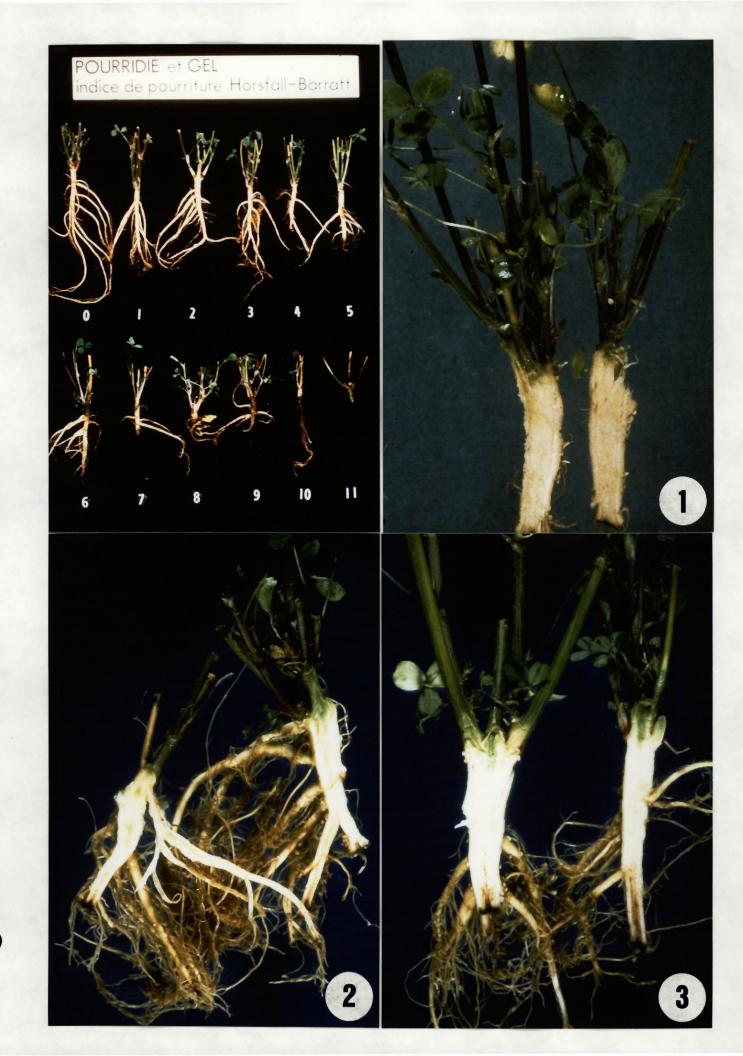


FIGURE 5 (Cont'd). Representation of the Horsfall-Barratt scale in alfalfa.



FIGURE 5 (Cont'd). Representation of the Horsfall-Barratt scale in alfalfa.



## III.4.3 Classification of the genotypes

Each genotype was classified according to its degree of response to inoculation with <u>Fusarium</u> root rot: resistant (R), intermediate (I), or susceptible (S). Table 5 illustrates the relationship existing between the classification system and the scale of the root rot evaluation.

# III.4.4 Statistical design and analysis of the parental population

The 450 red clover propagules were inoculated in six batches, every 5 days between June 17th and July 12th, 1982. Assessment of root rot disease began July 15th and ended August 9th, 1982 (Table 6).

The experimental design used was a 2 x 3 factorial in a randomized complete block design (RCBD). The two red clover cultivars, Arlington and Florex, were randomized equally within each inoculation block along with the three  $\underline{F}$ . isolates (814, 927 and 959). Because of the lack of space, block numbers 1, 2 and 3 were grown in a different greenhouse than block numbers 4, 5 and 6. The layout of the experimental plots is illustrated in Figure 6.

Before carrying any analysis on the data, I verified if the measured infection length (IL) was normally distributed, since this is one of the assumptions of the ANOVA analysis. The Kolmogorov-Smirnov test of normality given by the PROC Univariate of SAS (S.A.S. Institute, 1982) yielded mixed results where, in most cases, IL was not normally distributed (see APPENDIX 3). Deviations from the normality were, however, slight, ranging from 8 to 15%. A square root transformation of the data helped decrease these deviations from the normality, although some data was still not yet normal. It was felt that this correction was sufficient as the analyses to be carried were mostly correlations and chi-squares, which are apparently not effected as much by deviations from normality.

TABLE 5. Classification of the genotypes according to their response to <a href="Fusarium">Fusarium</a> root rot using two scales of measurement.

CLASSIFICATION	LENGTH OF INFECTION FROM INOCULATION SITE	HORSFALL- BARRATT SCALE
RESISTANT	less than 5 cm	0-1-2
INTERMEDIATE	between 5 and 25 cm	3,,8
SUSCEPTIBLE	more than 25 cm	9-10-11

TABLE 6. Date of inoculation, number of plants inoculated within each red clover cultivar, and the date of root rot assessment of <a href="Eusarium">Fusarium</a> root rot on the genotypes.

BLOCK Number	DATE OF INOCULATION	NUMBER OF PLO ARLINGTON	ANTS/CULTIVAR FLOREX	DATE OF ROOT ROT ASSESSMENT
1	82.06.17	36	36	82.07.15
2	82.06.22	36	36	82.07.20
3	82.06.27	36	, 39	82.07.25
4	82.07.02	36	36	B2.07.30
5	82.07.07	36	36	82.08.04
6	82.07.12	36	26	82.08.09

FIGURE 6. Layout of the experimental blocks of the parental plants.

date: July 02 July 07 July 12 427 files from Land William was 422 and 443 files BLOCK BLOCK 6 BLOCK 5 June 27 date: June 22 June 17 BLOCK BLOCK 1 BLOCK

The results of the analysis of variance (ANOVA) of the parental clone red clover populations is given in Table 7. Significant differences were found between the groups, the F. isolates and the interaction between the F. isolates and the cultivar. The nonhomogeneity found among the groups was indicated by a slight increase in mean E. infection length along the light gradient (Figure 6; Table 8). But since the two red clover cultivars and the three F. isolates were equally distributed among the groups, we can assume that the length of infection increased in a similar manner within the two red clover cultivars, and the three Fusarium isolates, thus not biaising the data.

# III.5 INHERITANCE ANALYSIS OF RESISTANCE TO FUSARIUM ROOT ROT III.5.1 Genotypes\_used

Plants to cross were chosen according to the reaction of their genotypes to <u>Fusarium</u> infection. Genotypes were classified as being resistant (R), intermediate (I) or susceptible (S) to either one or two races of <u>Fusarium roseum</u>. It was intended to make specific crosses and their reciprocals in all possible combinations between and within the two red clover cultivars. Unfortunately, the greenhouse space was not available to grow all the progeny which would have been generated from this number of crosses.

Instead, specific crosses within each cultivar included the following categories: resistant by resistant; resistant by susceptible; and susceptible by susceptible. Approximately 40 plants were obtained for each reciprocal cross (Table 9).

Additional crosses were done within the cultivar Arlington. They were: resistant by intermediate; intermediate by intermediate; and intermediate by susceptible (Table 9).

TABLE 7. Analysis of variance of the parental red clover populations.

Source	df	Type I SP SS	F value .	PR > F
Group	5	86.5	7.68 **	0.0001
Cultivar	1	3.5	1.56	0.2122
E. isolate	3	95.1	14.08 **	0.0001
Cultivar x Isolate	3	41.8	6.18 **	0.0005

<sup>\*\*</sup> Significant at the 0.01 level

TABLE 8. Mean infection length (square root transformed) among the inoculation groups of parental red clover populations (without control plants).

Group	N	Mean
6	62	4.3 a
5	72	3.8 b
4	72	3.4 bc
i	72	3.3 bc
2	72	3.0 c
3	72	3.0 c

<sup>\*</sup> Means with different letter are significantly different (P < 0.05) according to the Duncan's multiple range test.

TABLE 9. Number of plants obtained from each category of cross classified according to its degree of resistance to two races of <u>F. roseum</u>.

RED CLOVER CULTIVAR			ARLIN	GTON			FLOREX	Į.
		R	I	S		R	I	S
	R	40	10	20		40		20
ARLINGTON	I	10	40	10	FLOREX		20	
	S	20	10	40		20		40

<sup>\*</sup> Resistance category: resistant (R)
intermediate (I)
susceptible (S)

#### III.5.2 Crossing techniques and seed production

III.5.2.1 ENVIRONMENTAL CONDITIONS. All crosses were made in a greenhouse. The first set was carried out between August and October 1982, under natural light and temperature conditions. Plants were covered by a fine white net to keep bumblebees and other pollinating insects out of reach of the flowers. The second set of crosses was carried out from February to April 1983. Artificial sodium lights were used to extend light period to 16 hours per day. Temperature was kept at 22°C day, 18°C night.

III.5.2.2 PLANT PREPARATION AND POLLINATION. Plants to be crossed were supported by a wire frame. Reciprocal crossing was done by hand without emasculation. Once the faded flower heads were removed from the plant, each remaining inflorescence was separated using a forceps into three parts of about 10 florets each. Only half-opened flowers were retained.

Two instruments were tested to collect pollen. The first one involved the use of a folded piece of white paper. The second instrument consisted of a toothpick, to which black velvet was glued at the tip (Figure 7). The pollinating instrument was inserted between the standard and the keel. Downward pressure applied on the latter caused the staminal column to strike the instrument. For reciprocal crosses, the pollen was collected from approximately 5 florets and then applied alternatively between paired sections of heads of different plants using the same pollinating instrument.

Selfing one or two heads per plant was achieved by pollinating each flower of the same section twice. This was done to verify the hybrid nature of the seeds. And since no seeds came out of these crosses, all the others were assumed to be hybrid. After all flowers of a particular

FIGURE 7. Hand-pollination in red clover using a toothpick covered with black velvet.

FIGURE B. Identification of the crosses made in red clover.





cross had been pollinated, a small tag was looped and secured over the stem immediately under the head. Tags were labelled as to parentage and crossing dates with an indelible pencil to prevent loss of the record when the plants were watered (Figure 8).

III.5.2.3 HARVESTING AND STORAGE. Fertilized ovaries were allowed to mature six weeks before harvesting. The whole flower head was then cut. One week later, seeds were removed from the remnant calyx by rubbing with the fingers. The first batch of seeds were kept at room temperature, while the second set was stored in a refrigerator at about 10 degrees C.

## III.5.3 Progeny evaluation

The entire progeny of a particular cross was inoculated with the most virulent source of fungus that was used to classify the reaction of its parents. The application technique (AT), previously described, used to inoculate the clones, served to induce the disease in the progeny. The F1 progeny was inoculated with <u>Fusarium roseum</u> isolate 814 on June 17th, 1984, when 8-month old. They were evaluated for intensity of <u>Fusarium</u> root rot infection four weeks later.

# III.5.4 Statistical design and analysis of the progeny population

No particular statistical design was used for the second batch of progeny. The use of sodium lights reduced the light gradient which could have altered our data.

Testing of the normality of IL measured on the progeny was done. The progeny also required transformation of the data by using their square root. But since the original IL and its square root were strongly correlated in both cultivars (0.97 in Arlington, and 0.96 in Florex). The results obtained were the same when the analysis was carried out

without any transformation.

III.5.4.1 PARENT-PROGENY CORRELATIONS AND REGRESSIONS. Pearson correlation coefficients were calculated between the <u>Fusarium</u> infection length of the progeny and the mean <u>F</u>. infection length of their parents, for each cultivar. The assumption behind this analysis is that the more heritable is the resistance to <u>Fusarium</u> root rot, the closer to one should the correlation coefficient be. These correlation coefficients can be considered as estimates of narrow sense heritability (Frey and Horner, 1957). Similarly, a regression slope was fitted to the above data in order to verify the fit of the relationship. These analyses were carried out using SAS procedures CORR and GLM (SAS Institute, 1982).

FUSARIUM ROOT ROT. Pearson correlation coefficients were computed for the progeny population tested between <u>Fusarium</u> root rot infection length and the following morphological characters: the width of the crown, the width of internal breakdown, the regrowth of the foliage and the dry weigth of the inoculated root system.

# IV. RESULTS AND DISCUSSION

- IV.1 DEGREE OF SUSCEPTIBILITY TO FUSARIUM ROOT ROT IN THE RED CLOVER SOURCE POPULATIONS
- IV.1.1 Comparison of the two methods of measuring root rot susceptibility

The use of the Horsfall-Barratt scale for assessing <u>Fusarium</u> root rot susceptibility gave rise to results very similar to those obtained with the measurement of the infection length. The major differences, observed between the two methods lie in a numerically greater proportion of resistant genotypes and a smaller proportion of susceptible genotypes with the use of the Horsfall-Barratt scale in the parental population (Table 10 vs Table 11). A high degree of correlation (0.87 in Arlington and 0.88 in Florex, both significant at 0.001 level) between the results obtained with the Horsfall-Barratt scale and those obtained when measuring the length of infection from the inoculation site were found. A chi-square test of independence between the proportion of resistant, intermediate and susceptible classes of genotypes obtained by the two methods did not show any significant difference at the 0.05 level.

A similar trend was observed between the two methods of disease assessment in the progeny population (Table 12). In this case, however, the use of IL gave rise to a numerically greater proportion of susceptible plants over the two red clover cultivars. The proportion of resistant genotypes was higher with the use of IL than HB scale in Arlington, whereas the proportion of resistant genotypes was higher with HB than IL in Florex. The correlation between the results obtained with

TABLE 10. <u>Fusarium</u> root rot level of susceptibility of a red clover population, composed of the cultivars Arlington and Florex, as measured by the infection length from the inoculation site.

Level of susceptibility	<u>Fusar</u> 814		<u>rium</u> isolate n 927 9			number 959 Total		
Resistant (< 5cm) Intermediate Susceptible (> 25cm)	86	(20%) (61%) (19%)	74	(32%) (53%) (14%)	63	(35%) (45%) (21%)	223	(29%) (53%) (18%)
Total	142	(100%)	139	(100%)	141	(100%)	422	(100%)

TABLE 11. <u>Fusarium</u> root rot level of susceptibility of a red clover population, composed of the cultivars Arlington and Florex, as measured by the Horsfall-Barratt scale.

Level of susceptibility	<u>Fusarium</u> isolate number								
	1	B14	(	727	959	7	Tota	al	
Resistant (0-1-2)	33	(23%)	55	(40%)	55	(39%)	143	(34%)	
Intermediate	88	(62%)	70	(50%)	66	(47%)	224	(53%)	
Susceptible (9-10-11)	21	(15%)	14	(10%)	19	(14%)	54	(13%)	
Total	142	(100%)	139	(100%)	140	(100%)	421	(100%)	

TABLE 12. <u>Fusarium</u> root rot level of susceptibility of the progeny red clover population, inoculated with <u>F.</u> isolate 814, within Arlington and Florex using two methods of inoculation.

Method of	Arli	ngton	, Elo	rex	Total			
inoculation Susceptibility	IL	нв	IL	нв	IL	нв		
Classes	(%)	(%)	(%)	(%)	(%)	(%)		
Resistant	33 (14)	24 (10)	33 (14)	37 (15)	66 (14)	61 (13)		
Intermediate	155 (65)	176 (74)	181 (75)	187 (78)	336 (70)	363 (76)		
Susceptible	49 (21)	37 (16)	26 (11)	16 (7)	75 (16)	53 (11)		
Total (100%)	237	237	240	240	477	477		

N.B. Data calculated over the following crosses: R x R, R x S, S x S.

HB and IL in the progeny was very high (r=0.88 \*\* in Arlington; r=0.91 \*\* in Arlington). A chi-square test of independence between the proportion of resistant, intermediate and susceptible classes of genotypes obtained by the two methods did not show any significant difference at the 0.05 level.

The two methods appear redundant since they seem to measure the same thing. Nevertheless, both methods may be usefull when assessing the extent of <a href="Eusarium">Eusarium</a> root rot. The length of infection from the inoculation site (IL) could measure the capacity of the fungus to progress along the vascular bundles, whereas the Horsfall-Barratt scale could indicate its ability to completely propagate and induce rotting of the root tissues. Richard <a href="Eusarium">Eusarium</a> root rot in alfalfa, used a combined index, by multiplying the results obtained with the two methods. However, several pathologists are using the length of infection from the inoculation site (Leath and Kendall, 1978; Pederson <a href="Eusarium">Et al.</a>, 1980).

Since the analysis of our results obtained with both scales of measurement did not reveal any different information, it was decided to use the length of infection for the purpose of discussion. Having decided upon the most appropriate scale of measurement, we can proceed to analyze the degree of susceptibility of the sample population to <u>Fusarium</u> root rot.

## IV.I.2 Degree of susceptibility of sample populations of the cultivars Arlington and Florex to Fusarium root rot

The results obtained over the entire red clover parental population tested are summarized in Table 13. Four hundred and twenty-two (422) genotypes were inoculated with the pathogen causing <a href="Fusarium">Fusarium</a> root rot. Among them, 216 belonged to the cultivar Arlington, while 206 were from

TABLE 13. <u>Fusarium</u> root rot level of susceptibility of Arlington and Florex genotypes, as measured by the length from the inoculation site averaged over the three <u>Fusarium</u> isolates.

Level of susceptibility	Cultivar			Parental population	
	Arli	ington	Florex		Mean
•	#	(%)	#	(%)	# (%)
Resistant (less than 5cm)	66	(31)	57	(28)	123 (30)
Intermediate	112	(52)	111	(54)	223 (53)
Susceptible (over 25cm)	38	(18)	38	(19)	76 (18)
Total	216	(100)	206	(100)	422 (100)

TABLE 14. Mean infection length (mm) in Arlington and Florex red clover genotypes inoculated with three isolates of <u>Fusarium roseum</u>.

E. roseum RACE	N	MEAN	Duncan's Multiple Control included	Range Test excluded
814	142	16.0	a	a
959	141	14.2	a	ab
927	139	12.9	a	b
CONTROL	28	4.8	b	-

Means with the same letter are not significantly different at 0.05.

the cultivar Florex. Based on an arbitrary classification from infection length results, the source population was composed of 29% resistant, 53% intermediate and 18% susceptible genotypes to <a href="Eusarium">Eusarium</a> root rot (Table 13). These results show that red clover appears moderately susceptible to <a href="Eusarium">Eusarium</a> root rot pathogen. A large proportion of clones have an intermediate susceptibility which would probably not kill the plants in the field but weaken them to the point that they may not survive harsh winter conditions and be more susceptible to other diseases. This large proportion of intermediate susceptibility may be linked to the disappearance of red clover under normal field conditions. Similar, results were reported in the literature but under field evaluation of <a href="Eusarium">Eusarium</a> root rot.

The segregation of each class within each cultivar, averaged over the three <u>Fusarium</u> isolates, was quite similar (Table 13). Because of its slightly greater percentage of resistant genotypes and its lower percentage of susceptible genotypes, Arlington appears more resistant than Florex to <u>Fusarium</u> root rot disease. However, a chi-square test of independence between the cultivars and susceptibility classes indicates that the two populations are not significantly different at the 0.05 level with respect to susceptibility.

## IV.1.3 Comparison of the virulence of Fusarium races on red clover cultivars

The virulence of each <u>Fusarium</u> isolate varies with the red clover cultivar used. A closer look at the segregation obtained within Arlington and Florex cultivars, for each of the <u>Fusarium</u> isolates used, shows some differences in the percentage of plants belonging to each of the category of susceptibility.

The mean length of Fusarium infection measured from the inoculation

genotypes is illustrated in Table 14. The 28 control plants had an infection length mean of 4.8 mm, showing a significant difference (at 0.05 level) from the infection length of inoculated genotypes. However, no significant differences were observed when comparing the mean infection length of each <a href="Eusarium">Fusarium</a> isolate. Isolate #814 had the longest mean infection length (16.0 mm), averaged over the 142 genotypes, followed closely by isolate #959 with an infection length of 14.2 mm (over 141 genotypes). The isolate #927 caused a mean infection length of 12.9 mm (over 139 genotypes). Globally, <a href="Eusarium">Fusarium</a> roseum isolate #814 is the most virulent on our test plants while isolate #927 is the least virulent, with isolate #959 occupying an intermediate position.

IV.1.3.1 VIRULENCE OF <u>FUSARIUM</u> <u>ROSEUM</u> ISOLATES ON CULTIVAR ARLINGTON. A total of 216 genotypes from Arlington were inoculated with one of three races (Table 15). The <u>Fusarium</u> isolate #959 induced the longest mean infection length (16.3 mm), while the isolate #814 caused a mean infection length of 13.9 mm, closely followed by the isolate #927 with 13.6 mm. However, none of the treatments within the cultivar Arlington showed any significant difference at 0.05 level. However, numerically, isolate 959 appears the most virulent on Arlington.

Table 16 summarizes the proportion of Arlington plants falling into each class of susceptibility to the disease. The proportion of resistant Arlington phenotypes found with each pathogen ranges from 27% with isolate # 814, to 29% with # 959, up to 36% with race # 927. The percentage of susceptible phenotypes classified within each pathogen ranges from 15% (for both F. # 814 and F. # 927) up to 23% (F. # 959). Fusarium isolate 959 appears the most virulent on Arlington as evidenced by more susceptible and less resistant plants than the others. Isolate

927 appeared the least virulent while isolate 814 seems to be intermediate in this respect. A chi-squre test, however, shows that these trends are not strong enough to be significant at 0.05 level.

IV.1.3.2 VIRULENCE OF ISOLATES OF FUSARIUM ROSEUM ON CULTIVAR FLOREX. A different picture for the virulence of Fusarium isolates was obtained from the inoculations within the cultivar Florex (Table 15). Over 200 Florex plants were also inoculated with one of the three races of Fusarium. A significant difference was found among the inoculation treatments. Fusarium isolate #814 caused a significantly longer mean infection length (18.1 mm), compared to the mean infection lengths of isolates #927 and #959 (both with 12.2 mm of infection).

<u>Fusarium</u> isolate 814 appears to be the most virulent strain over the entire population (Table 14) and caused significantly longer infection length than the other two isolates in the cultivar Florex (Table 15).

Table 17 summarizes the proportion of plants belonging to each resistance category to the disease in the Florex cultivar. The percentage of resistant genotypes observed when inoculated with each pathogen varied from 13% (F. \* 814), to 29% (F. \* 927) up to 41% (F. \* 959). The proportion of susceptible genotypes ranges from 14% (F. \* 927), to 18% (F. \* 959), up to 23% (F. \* 814). In this case, contrary to that of the cultivar Arlington, a significant chi-square test demonstrated that <u>Fusarium</u> isolate \*959 was the least virulent while 814 the most virulent (Table 17). Isolate 927 caused an intermediate reaction in Florex. Therefore, the variability in the range of percentage of plants belonging to the resistant and susceptible categories is much more pronounced with the Florex than in Arlington genotypes.

TABLE 15. Mean infection length (mm) within the cultivars Arlington and Florex inoculated with three isolates of Fusarium roseum.

E. roseum RACE	ARL N	INGTON MEAN	F N	LOREX Mean	T-TEST BETWEEN CULTIVARS
B14	73	13.9 a	69	18.1 a	**
959	70	16.3 a	71	12.2 b	*
927	73	13.6 a	66	12.2 b	ns

Means with the same letter within the same column are not significantly different at 0.05 according to the Duncan's multiple range test.

- \*: means significantly different at the 0.05 level
- \*\*: means significantly different at the 0.01 level

TABLE 16. Reaction of Arlington genotypes to inoculation with three <a href="Eusarium roseum">Eusarium roseum</a> isolates as measured by the infection length from the inoculation site.

	FRANKIOW IZATE NOMBEK			
LEVEL OF SUSCEPTIBILITY	B14	927	959	
	Number of plants	(percentage (	over isolate)	
Resistant ( < 5 cm)	20 (27%)	26 (36%)	20 (29%)	
Intermediate	42 (58%)	36 (49%)	34 (49%)	
Susceptible ( > 25 cm)	11 (15%)	11 (15%)	16 (23%)	
TOTAL	73 (100%)	73 (100%)	70 (100%)	

TABLE 17. Reaction of Florex genotypes to inoculation with three <a href="Eusacium roseum"><u>Fusacium roseum</u> isolates as measured by the infection length from the inoculation site.</a>

	<u>FUSARIUM</u> ISOLATES NO.			
LEVEL OF SUSCEPTIBILITY	814	927	959	
	Number of plants	(percentage	over isolate)	
Resistant ( < 5 cm)	9 (13%)	19 (29%)	29 (41%)	
Intermediate	44 (64%)	38 (58%)	29 (41%)	
Susceptible ( > 25 cm)	16 (23%)	9 (14%)	13 (18%)	
TOTAL	69 (100%)	66 (100%)	71 (100%)	

IV.1.3.3 DISCUSSION OF THE VIRULENCE OF FUSARIUM ROSEUM RACES. The results obtained in this experiment differ slightly from those previously reported in the literature, but they agree with their general conclusions. The greatest virulence (with pooled results over all clones) was obtained with isolate #814, which agrees with results published by Tetteh (1980), the only data available on this Fusarium race. Tetteh (1980), using the application technique on 17 red clover accessions (10-day old seedlings), found that isolate #814 and #959 significantly reduced stands of the seedlings, when isolate #927 did not reduce stands differently from the control treatment. Isolate #814 was found more virulent than isolate #959.

To be consistent with the general statement that a host species is more severely attacked by its own isolates (Leath and Kendall, 1978), Fusarium isolates #814 and #959, which came from red clover, should be more virulent than isolate #927 which came from alfalfa (Table 4). Dur results agree with this statement, since isolates #814 and #959 induced a longer mean infection length than isolate #927 (see Table 14). Leath and Kendall (1978), in a pathogenicity and host range study, noticed some differences among Fusarium isolates of different host species in their ability to induce root rot diseases. They concluded that, in general, host species were more severely affected by isolates coming from the same species than by isolates coming from other related species, thus causing more important root rots on them. reported however, that some isolates were found more virulent on species from which they did not originate, but never was the virulence in these exceptional cases very high. Leath and Kendall (1978) using the slantboard technique, measured the length of root rot infection in the red clover cultivar Pennscott (4 days after inoculation) at two sites of

inoculation: (1) with the inoculum placed on the tip of the root; and (2) with the inoculum placed on the root 2 cm above the tip. They report that <u>Fusarium</u> isolate #927 caused root rot only when inoculated to the tip of the roots, but was ineffective when inoculated at 2 cm above the root tip. On the other hand, isolate #959 caused root rot at both inoculation sites. The authors concluded that the mode of action of the two isolates was different. Isolate #927 seemed unable to penetrate and cause root rot in intact roots when inoculated at sites other than the root tip, while isolate #959 possessed the ability to penetrate directly through the roots. When the fungus isolates were inserted in the root 4 cm below the crown (the application technique). Leath and Kendall (1978) found that <u>Fusarium</u> isolate #927 was more virulent (infection length: 19 mm) than isolate #959 (infection length: 14 mm) on 4-month old Pennscott seedlings after 3 weeks of inoculation. These results suggested that the pathogenicity of isolate #927 was limited by its ability to penetrate the roots. Similar results were obtained by Pederson et al. (1980) when working with Arlington red clover using the application technique. After three weeks of incubation, isolate #927 caused a greater amount of root rot (mean infection length: 11.8 mm) than isolate #959 (8.2 mm).

In the present study, there seems to be a difference in the ability of each isolate to induce rot infection between the two cultivars used in this experiment. A significant difference was found in the rate of the various susceptibility caused by isolates #814 and #959 between Arlington and Florex clovers (Table 15). <u>Fusarium</u> isolate 927 did not show any significant difference over the two cultivars used.

A direct comparison between the actual mean infection lengths of root rot obtained in the literature and the ones reported here should be made with caution. Even if the inoculation techniques were similar (the

application technique), the duration of the incubation period reported in the literature (3 weeks) was different from the period of 4 weeks used in this experiment. In addition, the preparation of the inoculum was also different. For instance, Leath and Kendall (1978), as well as Pederson et al. (1980), grew their <u>Fusarium</u> isolates on vegetable juice agar while PDA was used in the present study. Leath and Kendall (1978) exposed their isolates to the light, while in Pederson et al. (1980), as well as in the present study, isolates were grown in the dark. The exposure to the light may cause a more vigorous mycelial growth and the production of conidia and chlamydospores that might result in more severe infection. Using isolate 927, Leath and Kendall (1978) reported a, longer infection length in Pennscott (19 mm), compared to 11.8 mm in Pederson et al. (1980) with Arlington, and 13.6 mm with Arlington in the present study. The same tendency was observed with isolate #959: an infection length of 14 mm reported in Pennscott (Leath and Kendall, 1978) compared to only 8.2 mm obtained with Arlington by Pederson et al. (1980), and 16.3 mm in the present study. The conclusion that Arlington red clover would be more resistant than Pennscott to <u>Fusarium</u> root rot could be suggested, but since these two cultivars were not tested in the same experiment we cannot be certain of this. In our experiment, Arlington red clover showed a mean'infection length of 16.3 mm with isolate #959 and 13.6 mm with isolate #927 compared to 8.2 mm with isolate #959 and 11.2 mm with isolate #927 reported by Pederson et al. (1980). The difference observed between the two experiments can be partially attributed to the length of the incubation period, which was one week longer in our case. In addition, variable conditions for plant growth among the experiments would also cause differences in infection length.

### IV.2 DEGREE OF SUSCEPTIBILITY TO FUSARIUM ROOT ROT OF THE PROGENY DERIVED FROM SPECIFIC CROSSES

The progeny obtained from specific crosses between plants belonging to identified resistance categories were all inoculated with the same <a href="Eusarium">Fusarium</a> isolate: number 814. The response of these progeny to <a href="Eusarium">Fusarium</a> root rot was summarized according to the same classes as those used for their parents to allow a valid comparison between the two populations.

Overall the entire progeny population (477 plants), fourteen percent (14%) of the genotypes were classified as resistant, seventy percent (70%) intermediate and sixteen percent (16%) susceptible (Table 12).

The two cultivars reacted differently to the disease. A chi-square test of independance between the two populations indicated that they were significantly different at the 0.05 level. The percentage of resistant genotypes (14%) was identical for the two cultivars, but Arlington had a greater percentage of susceptible genotypes (21%) than Florex (11%). In the progeny population, Florex red clover appears more resistant to <u>Fusarium roseum</u> isolate B14 than Arlington.

In the parental population, Arlington had more resistant plants (27%) than Florex (23%) to <u>F.roseum</u> race 814. This may be due to the progeny being inoculated at an earlier stage of physiological maturity. Gene segregation may also explain this differential result.

#### IV.3. PARENT-PROGENY CORRELATIONS AND REGRESSIONS: INHERITANCE STUDY

The main objective of this project was to determine the inheritance of <u>Fusarium</u> root rot in red clover. The approach used to investigate this matter consisted of doing a series of single reciprocal crosses of plants within and between classes of <u>Fusarium</u> root rot susceptibility within each red clover cultivar.

Table 18 gives a description of the levels of susceptibility of parents involved in crosses and their progenies. Approximately forty plants were produced for each cross (Table 9).

### IV.3.1 Homogeneity obtained within each reciprocal crosses

Pooling of the reciprocal crosses should be done only if the population within each cross is proven to be homogeneous. The results of a chi-square test of independence and heterogeneity, made to test the latter, are given in table 18. Reciprocal differences were found within two of the crosses within Arlington red clover only. The two types of crosses concerned are the resistant x resistant and the resistant X susceptible ones.

Differences among reciprocal crosses could infer that the genetic transmission could be of a cytoplasmic nature. If the offspring were always like their maternal parent, it would suggest that the hereditary transmission is through the cytoplasm rather than the nucleus (Crow, 1976). Differences among reciprocal crosses have been reported in the literature for another quantitative character, namely the yield. Taylor and Smith (1979) reported the results of an Italian study by Ceccarelli (1971) where reciprocal differences for forage yield were found in red clover. Genetic variance was greater among populations than within. Additive genetic variance was a significant portion of the genetic variance among a diallel progeny from plants selected from wild and cultivated populations.

Hypotheses for inheritance were developed from the distribution of disease classes (R, I and S) from the F1 families from each gene pool (i. e. Arlington and Florex).

TABLE 18. Segregation of F1 progeny derived from specific crosses of parents selected from two Arlington and Florex populations for their susceptibility to Fusarium root rot.

		PARENTAL	GENOTYPES			F1 PROGENY	
Cultivar	cross	in the cross	Infection length (mm)	length (mm)	(R:I:S)	infection length (mm)	
ARLINGION	RXR	37 x 185	0 3		4:13:26	23.3	14.55 **
		20 X 29 29 X 20			5:36: 2 5:25:13		10.04 **
		74 X 135 135 X 74			15:29: 0 11:33: 1	9.9 9.8	1.85 ns
		185 X 206 206 X 185			3:15: 2 1:18: 4		2.63 ns
		1 X 6 6 X 1			4:32: 4 9:23: 6		2.70 ns
		6 X 28 28 X 6			3:11: 1 3:16: 1		0.62 ns
ELOREX	R X R R X R	269 X 270 270 X 269	3 2 2 3	2.5 2.5	12:29: 4 15:25: 3	10.0	0.73 ns
		277 X 275 275 X 277	4 26 26 4		4:35: 4 7:34: 3		0.95 ns
		279 X 294 294 X 279	50 30 30 50		3:33: 8 4:33: 6	21.1 18.7	0.58 ns

### IV.3.2 Analysis of F1 intrapopulation means

The intrapopulation mean of each type of cross varies in the opposite direction. In Arlington, progeny derived from crosses between resistant parents (R x R) are much more infected than progeny obtained from cross between susceptible parents (S x S). Whereas in Florex, progeny coming from the RxR type of cross gave rise to progeny less infected than those derived from the SxS type of cross. In both cultivars however, the RxS crosses occupied an intermediate position, being almost exactly in the middle of their respective means. In Arlington, the progeny mean of <u>Fusarium</u> infection length is slightly less than the middle value, while in Florex, the progeny mean is slightly above the mean value. The noninoculated controls of both cultivars gave rise to resistant plants only. The mean of their <u>Fusarium</u> infection length was significantly different from the treated plants.

It seems that these two populations may have different resistance mechanisms or perhaps the lack of progress in selection for resistance in Arlington indicates a lack of resistance genes in this cultivar. A larger number of crosses within and between cultivars would have to be made to distinguish between these possibilities. The occurence of different resistance mechanisms has been reported in the literature. In alfalfa, Viands et al. (1979) comparing the inheritance of resistance to bacterial wilt in two alfala gene pools found that the two populations studied had different resistance mechanisms. Their conclusion based on a quantitative analysis were further supported by qualitative analysis done by Viands and Barnes (1982).

Michaud and Richard (1986, personal communication) also reported that they obtained a different pattern of inheritance to <u>Fusarium</u> root rot while working with different populations of red clover.

### IV.3.3 Parent-progeny correlations and regressions

Pearson correlation coefficients calculated between the E. infection length of the progeny and the mean E. infection length of their parents are reported in Table 19. In both cultivars, the mean E. infection length of the parents is moderately correlated with the E. infection length obtained in the progeny. In Arlington, the correlation value of parent-progeny E. infection length is -0.49 (significant at the 0.0001 level), whereas in Florex, the same correlation value gave 0.37 (significant at the 0.0001 level). The overall population mean being negatively correlated at 0.10 (significant at the 0.02 level).

If we consider these correlation coefficients as estimates of narrow sense heritability (Frey and Horner, 1957), then we would have to conclude that the value is 0 for Arlington, indicating a lack of resistance genes in this cultivar. In Florex, resistance seems to have a low to moderate heritability, suggesting that progress could be made in selection for <u>Fusarium</u> resistance, providing progeny testing was done.

Regression analyses showed similar results to correlations (Table 19). The slope of the regression of parent to progeny infection length in Florex is 0.26 (r square= 0.94), while in Arlington the slope is negative at 0.38 with a r square of 0.90. These values are comparable since in each case, we are not considering crosses including parents with intermediate level of susceptibility. When we consider intermediate crosses that were made in Arlington, we obtained a lesser negative slope of 0.30 (r square= 0.52). When all the progenies of both cultivars are pooled together in the regression analysis, the slope is not different from zero with a very weak fit (r square= 0.01). This further supports the conclusion that the two cultivars possess different resistance mechanism to Fusarium root rot.

TABLE 19. Correlation between the parental mean  $\underline{F}$ . infection length (MIL) and that of their progeny (PIL) for each cross.

	Overall progeny	Arlington	Florex
Correlations MIL * PIL	r = -0.10388 p = 0.0236 n = 475	r = -0.49808 p = 0.0001 n = 235	r = 0.37056 p = 0.0001 n = 240
Regressions MIL * PIL	b = 0.035 r-sq = 0.0118 df = 16	b = -0.384 r-sq = 0.896 df = 4	b = 0.263 r-sq = 0.94 df = 4
		b = -0.299 (inc r-sq = 0.521 df = 10	Intermediate)

#### IV.4 OCCURRENCE OF INTERNAL BREAKDOWN

Throughout the experiments, while splitting the taproot of the plants longitudinally, the central area of the crown appeared necrotic, with empty spaces in some cases (Figure 9). This crown deterioration was identified as the result of internal breakdown (IB) first reported by Graham et al. (1960). Internal breakdown is considered by some as one of the major factors involved in the lack of persistence of red clover, particularly in Northeastern North America (Newton and Graham, 1960; Cressman, 1967).

The overall incidence of IB among the parental population was 40% in Arlington and 41% in Florex. In the progeny population, the occurence of IB dropped to 33% in Arlington and 27% in Florex, the overall mean of their population being 31%. These results corroborate that previously reported in the literature. Cressman (1967) found IB in 56% of the 405 red clover plants that he examined in a three month old stand. In a greenhouse study, Graham et al. (1960) found that the incidence of IB increased from 23% of the plants at the end of 12 weeks to 72% at the end of 41 weeks. All the cultivars of red clover they tested showed IB: Pennscott, Dollard, Midland and Lakeland. However, the severity and the time of appearance varied with the cultivar.

Graham et al. (1960) also reported that in the field, damage was more severe when IB was accompanied by weevil injury and root rot. The hypothesis that internal breakdown would induce weakness within the taproot and subsequently allow more root rot to develop would favor a positive correlation between IB and root rot incidence.

In the present study the width of IB at the crown level was measured in plants showing this breakdown in the progeny. Pearson correlation coefficients between <a href="Fusarium">Fusarium</a> root rot infection length

FIGURE 10. Internal breakdown in red clover (<u>Trifolium pratense</u> L.).



(IL), the width of the crown (CW) and the width of internal breakdown (IBW) were calculated for the progeny derived from the R x R, R x S, and S x S crosses of Arlington and Florex red clover cultivars (Table 20). Results obtained within each cultivar did not differ from those calculated over the entire population. IL is negatively correlated with IBW (r=0.12364, p=0.0012). IL is negatively correlated with CW (r= 0.2010, p=0.0001). CW is correlated with IBW (r=0.39045, p=0.0001). According to these results, the length of <u>Fusarium</u> root rot infection decreases as the occurence of IB increases and the width of the crown increases. IBW increases as the crown diameter increases. Cressman (1967) demonstrated a direct association between the % of internal breakdown infection within the taproot and the enlargment of the crown. A graphical representation of my results show the same pattern (Figure 10), but with less precision. On this Figure, the darker squares indicate a higher number of data points at the same coordinates. The IB seems to appear when the crown diameter reaches 9 mm.

The exact cause of IB is not well understood. In 1960, Graham et al. suggested that it could indirectly be due to the effect of a missing unidentified minor-element. Histological studies made by Cressman (1967), failed to associate IB with any pathogen. He describes the disease as being a physiological disorder associated with the fast enlargement of the crown as the red clover plant ages.

## IV.5 EFFECTS OF MORPHOLOGICAL FACTORS ON SUSCEPTIBILITY TO FUSARIUM ROOT ROT

#### IV.5.1 In the parental population

All correlations involving crown diameter, top regrowth dry weight (after 31 days) and root dry weight were highly significant (Table 21), showing a positive association between traits that measure general vigor

TABLE 20. Pearson correlation coefficients between <a href="Fusarium">Fusarium</a> root root infection length, the width of the crown, the width of internal breakdown, the regrowth of the foliage, and the weight of the inoculated root system, calculated for the progeny population tested.

•	Overall progeny	Arlington	Florex
INFECTION LENGTH	0.01/0**	0.1/01**	0 740/ "
vs CROWN WIDTH	-0.2168**	-0.1681**	-0.3406**
VS CRUWN WIDTH	p=0.0001	p=0.001	p=0.0001
INFECTION LENGTH	0.1259	0.072B*	0.2927*
vs INTERNAL BREAKDOWN	p=0.0777	p=0.0403	p=0.0199
VS INTERNAL DICERDOWN	p-0.0///	p-0.0403	p-0.0177
INFECTION LENGTH	-0.2995**	-0.3079**	-0.2965**
, vs TOP REGROWTH	p=0.0001	p=0.0001	p=0.00B
	<b>F</b>	F	p
INFECTION LENGTH	-0.1379**	-0.1027	-0.2503*
vs ROOT WEIGHT	p=0.006	p=0.069	p=0.026
COOUN SIRIU	0.4848	A 4455	
CROWN WIDTH	0.1962**	0.1422*	0.4204**
vs TOP REGROWTH	p=0.0001	p=0.012	p=0.0001
CROWN WIDTH	0.5133**	0.4806**	0.7134**
vs ROOT WEIGHT	p=0.0001	p=0.0001	p=0.0001
	F ******	p 0.000.	p-010001
CROWN WIDTH	0.2692**	0.1822*	0.5849**
vs INTERNAL BREAKDOWN	p=0.001	p=0.035	p=0.001
INTERNAL BREAKDOWN	0.3205**	A 7710**	0.7404
vs TOP REGROWTH	p=0.001'	0.3718** p=0.000	-0.3106
VS TOT REDROWIN	p-0.001	p=0.000	p=0.353
INTERNAL BREAKDOWN	0.2848**	0.3059**	0.3484
vs ROOT WEIGHT	p=0.002	p = 0.002	p=0.294
	•	F	F
TOP REGROWTH	0.3123**	0.3260**	0.4429**
vs ROOT WEIGHT	p=0.000	p=0.000	p=0.000

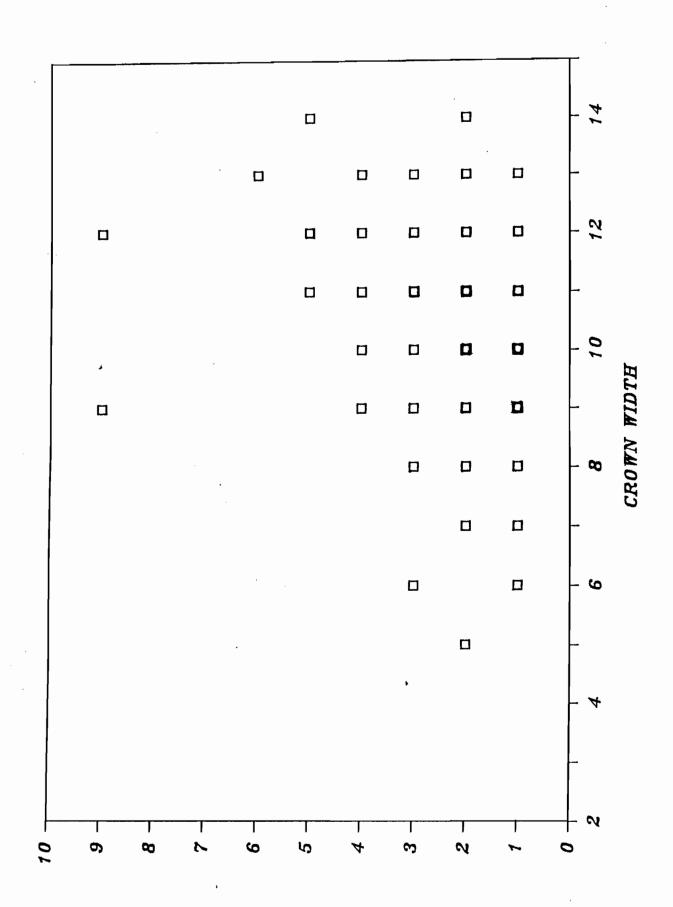
<sup>\*:</sup> significant at the 0.05 level \*\*: significant at the 0.01 level ns: not significant at the 0.05 level

of individual plants. Crown diameter was positively correlated with root dry weight (r = 0.34\*\*), indicating that plants with larger crown diameter tend to weigh more, therefore to have larger roots. Heavier top regrowth is also associated with heavier roots (r = 0.46\*\*). These highly correlated morphological characters agree with results reported by Pederson et al., 1980.

Our data, however, do not clearly establish an association between the root morphological characters and root rot incidence (Table 21). For example, root dry weight is not significantly associated with a reduced root rot infection in both red clover populations. Actually we obtained different significant correlations in the test populations of the two cultivars that are difficult to explain. In Arlington, only top regrowth dry weight negatively associates with root rot incidence (r = -0.14\*\*), while in Florex it is the crown diameter which is negatively correlated with root rot length (r = -0.16\*\*). These results suggest that plants with more vigorous top growth in Arlington are less prone to root rot, while in Florex the plants growing larger crown seem to suffer less from root rot.

These results appear contrary to some published results. Pederson et al. (1980) reported a significant correlation (0.22\*\*) between the length of root rot infection and root diameter in Arlington clover, concluding that plants with large root diameter tended to rot more than plants with small root diameter. Dur data do not support this conclusion. The authors do not specify in their study where the measure was taken on the taproot. A positive correlation between root rot and crown diameter indicates that plants with a large root diameter tend to suffer more from root rot than individual plants with thinner roots. A negative correlation would indicate the opposite: plants with small root

FIGURE 11. Correlation between internal breakdown and the width of the crown in the progeny



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TABLE 21. Correlation coefficients between root rot, crown diameter, top regrowth and crown diameter, top regrowth dry weight, root dry weight, for Arlington and Florex red clover cultivars, in the parental population.

CHARACTERS	!	ARLINGTON		FLORE	X	
	Crown diameter	Top regrowth dry weight	Root dry wt	! Crown ! diameter	Top regrowt dry weight	
ROOT ROT	0.09ns	-0.14**	0.03ns	:     -0.16**	-0.04ns	-0.06ns
CROWN DIA- METER (mm)		0.21**	0.34**	· ; ; ; ;	0.23**	0.35**
TOP RE- GROWTH (g)			0.46**	'     		0.56**

\*\*: significant at the 0.01 level ns: not significant at the 0.05 level

diameter tend to be more affected by root rot than plants with larger root diameter. Furthermore, Pederson et al. (1980) found that the correlation between root rot and plant dry weight (0.02) was not significant, but the negative correlations between root rot and regrowth (-0.15\*\* and -0.09\*\*) were significant. They concluded that susceptibility to F. roseum root rot was not associated with general vigor (for instance: plant dry weight) before inoculation, but infected plants had reduced vigor in their growth. Selection for root rot resistance would tend to reduce root diameter, but further research would be needed to determine the implications of this for field performance of root rot resistant red.clover.

### IV.5.2 In the progeny population

Pearson correlation coefficients were computed between the different morphological characters measured in the progeny population (Table 20). All correlations found within each cultivar, except one, indicates the same trend found in the overall progeny population.

The length of infection of <u>Fusarium roseum</u> race 814 was negatively correlated with the three following characters: a. the width of the crown (r=-0.22 \*\*); b. the regrowth of the foliage (r=-0.30 \*\*); and c. the weight of the infected roots (r=-0.14 \*\*). Greater infection length of <u>F.</u> race 814 is associated with smaller crown width and lighter roots. Similarly, the regrowth of the plant decreases as the length of <u>F.</u> infection increases.

Regrowth of the aerial part of the infected plants is positively correlated with the enlargment of the crown (r=0.20~\*\*) and the width of internal breakdown (r=0.32~\*\*). And in turn the width of internal breakdown is correlated with the width of the crown (r=0.27~\*\*). These results support those already found in the parental population and in

the literature (Peterson et al.,1980). Logically, top regrowth should be correlated with enlargment of the crown, since plants with large crown width are recognized to accumulate more carbohydrates in their root system. Our data support this theory since top regrowth and root weight are correlated (r= 0.31 \*\*). Cressman (1967) found that the incidence of internal breakdown increases as the width of the crown increases.

#### V. CONCLUSION AND SUGGESTIONS FOR FURTHER RESEARCH

Two sample populations from the red clover cultivars Arlington and Florex, were studied for their reaction to <u>Fusarium</u> root rot disease. Three isolates of <u>Fusarium</u> roseum (814, 927 and 959) were inoculated using the application technique to different genotypes to assess their virulence on red clover taproots and to identify the red clover genotypes showing resistance to <u>Fusarium</u> root rot.

Over the entire population, <u>Fusarium roseum</u> 814 was the most virulent isolate, inducing a mean length of infection of 16.0 mm, followed by <u>F. roseum</u> 959, with 14.2 mm, and <u>F. roseum</u> 927, with 12.9 mm. The genotypes classified as resistant (less than 5 mm of infection) made up 30% of the population, while the susceptible ones (more than 25 mm of infection) made up 18% of the population. The majority of the genotypes (52%) belonged to the intemediate category, their level of infection ranging between 5 and 25 mm.

The virulence of  $\underline{F}$ . isolates varied within each cultivar. Isolate 814 produced the longest mean infection in Florex (18.1 mm), whereas isolate 959 was the most virulent in Arlington (16.3 mm). Isolate 927 was the least pathogenic isolate over the two cultivars.

Specific crosses were made within each cultivar between genotypes classified as resistant (R), intermediate (I) and susceptible (S). The progeny obtained were inoculated with  $\underline{F}_{\perp}$  roseum 814 using the same inoculation technique used with their parents.

Within the progenies of these crosses, <u>F</u>. isolate 814 showed somewhat more pathogenicity on Arlington than on Florex. The proportion of resistant genotypes obtained was identical for the two cultivars

(33%), while the proportion of susceptible genotypes was much higher in Arlington (21%) than in Florex (11%).

The segregation obtained within each class of resistance within each type of cross was strikingly different for each cultivar. In Florex, the R x R cross gave rise to plants with less infection than for those derived from S x S crosses, while the opposite was found in Arlington. The narrow-sense heritability was estimated to be 37% in Florex, while in Arlington, the heritability estimate was 0, probably indicating the absence of genes for resistance in this cultivar.

Complementary data were taken during the experiments to relate morphological characters with <u>Fusarium</u> root rot. The occurrence of internal breakdown (IB) was noted in about 40% of the parental genotypes. In the progeny, its occurrence increased as the crown diameter increased, the critical diameter being 9 mm. Increased <u>Fusarium</u> infection was correlated with smaller crown diameter, lower top regrowth, and lower root dry weight.

The somewhat limited data of this study indicate that the inheritance of resistance to <u>Fusarium</u> root rot is quite complex showing low heritability. For further study, the procedures used in this study could be improved in the following ways:

- a. the inoculum could be composed of a culture of mixed <u>Fusarium</u> isolates. However, this would require the testing of the reaction of these fungi when they are grown together to verify that they are not antagonists;
- b. the plants should all be inoculated during the same day, and the assessment of their reaction to the disease completed on the same day. This would gave rise to more reliable results, as the plants would have exactly the same physiological age;

- c. it should be verified that the reaction of vegetative propagules to <u>Fusarium</u> infection is similar to the reaction of the parent plants. Plants reproduced vegetatively have a different physiological age than the plants obtained directly from the seeds;
- d. the design of the experiment could perhaps be improved. The suggested experimental design would be a split-split plot design, where the cultivar would be attributed to the main plot, and the <u>Fusarium</u> isolate to the subplot, if individual isolates are used;
- e. A greater number of crosses within and between resistance classes and cultivars should be done to verify the pattern of inheritance of resistance.

An alternative to this procedure would be the following. Prescreening of the genotypes could be done with at least three isolates of <a href="Eusarium">Fusarium</a> using the slant-board technique, described by Leath (1978). Mass screening could be achieved with the use of the gnobiotic chamber. Vegetative reproduction of the genotypes prior to inoculation should be done and crosses made between plants belonging to different classes of resistance.

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### VII.1 APPENDIX 1

# RED CLOVER GENOTYPES RESPONSE TO THREE FUSARIUM RACES

COLUMN	VARIABLE	DESCRIPTION
1	CLN	GENOTYPE NUMBER (MOTHER.GENOTYPE)
2	FUNGUS	FUSARIUM ISOLATE NUMBER (0 is control)
3	BLOCK	BLOCK NUMBER (1 to 6)
4	IL	ROOT INFECTION LENGTH, in mm
5	IC	DISTANCE OF UNINFECTED TISSUES FROM
		INFECTION SITE TO CROWN, in mm
6	нв	HDRSFALL-BARRATT SCALE
7	RE	DEGREE OF RESISTANCE (R, I or S)
. 8	RATIO	IL / (IC + IL)
9	IB	INTERNAL BREAKDOWN (Y or N)
10	CM	CROWN WIDTH, in mm
11	TAPLEN	TAPROOT LENGTH, in mm
12	SR	NUMBER OF SECONDARY ROOTS (more than 2 mm in dia.)
13	SRL	ADDED LENGTH OF SECONDARY ROOTS, in mm
14	PLANTWT	TOTAL PLANT DRY WT, in g (including taproot wt)
15	ROOTWT	TAPROOT DRY WEIGHT, in g

r	n		11	M	M	N	n	
L	U	L.	U	rı	14	N	U	

GENOT'	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.1	814	2	6	30	3	I	0.1667	Υ	6	30	11	36	8.06	1.33
1.2	927	3	17	23	3	I	0.4250	N	9	22	6	50	9.68	2.79
2.1	959	5	25	22	6	S	0.5319	N	8	47	0	0	5.18	1.86
2.2	814	5	18	17	4	1	0.5143	N	11	23	13	12	•	•
3.1	927	1	7	33	2	I	0.1750	Υ	5	40	0	0	8.87	2.74
3.2	959	1	5	17	2	R	0.2273	N	14	22	0	0	8.08	1.65
4.1	814	6	17	13	7	I	0.5667	N	7	30	0	0	5.95	2.58
4.2	927	6	30	3	10	S	0.9091	N	7	33	0	0	5.22	1.65
4.3	959	6	13	10	5	I	0.5652	N	11	23	4	23	3.25	1.42
5.1	814	4	5	40	2	R	0.1111	N	10	3B	10	45	10.29	2.69
5.3	959	3	7	33	5	I	0.1750	Υ	7	31	14	40	7.02	2.16
6.1	814	2	6	29	2	I	0.1714	Y	9	29	6	35	2.33	0.72
6.2	927	3	2	33	2	R	0.0571	Y	8	35	0	0	10.50	1.16
7.1	959	3	38	2	9	5	0.9500	N	6	40	0	0	5.82	1.03
8.2	927	3	3	32	2	R	0.0857	Y	10	21	6	35		•
8.3	959	3	2	28	1	R	0.0667	Y	6	25	10	30	5.15	0.98
9.1	814	5	16	34	4	I	0.3200	Y	9	20	8	50	8.01	2.58
9.2	927	5	18	22	4	I	0.4500	N	12	15	20	40	9.61	3.46
10.1	814	5	19	13	4	I	0.5938	Y	8	16	15	32	2.65	1.05
10.2	814	5	33	10	7	S	0.7674	N	5	12	4	43	2.29	0.57
11.1	959	6	6	69	2	I	0.0800	N	10	35	21	40	11.88	2.43
12.1	927	6	28	12	4	S	0.7000	N	9	18	16	40	5.73	3.10
13.2	927	4	13	27	4	I	0.3250	N	11	40	0	0		. 74
13.3	959	1	6.	24	2	I	0.2000	Y	12	30	0	0	5.61	0.71
14.1	814	1	9	36	3	I	0.2000	N	9	45	0	0	6.26	1.74
15.1	927	1		12	7	I	0.6000	N	9	30	0	0	B. 24	2.17
19.2	959	3	30 3	10	5	S	0.7500	N	9 7	10	14	40	4.08	1.28
20.2	814 927	4 5	36	27 10	2 4	R S	0.1000	N Y		30	.0	0	2.84	0.48
21.2	814	5	36 9	25	3	I	0.7826 0.2647	N	13 8	6 23	14	46 34	14.31 3.48	5.34
22.2	927	5	6	40	2	I	0.1304	N	10	16	18	46	8.38	1.39 1.85
23.1	959	4	32	4	10	S	0.1304	N	12	36	- 0	0	10.83	2.43
25.1	814	2	9	21	4	I	0.3000	N	9	30	0	Ö	1.95	1.07
26.1	927	2	3	37	2	Ŕ	0.0750	, Å	7	24	13	40	5.47	1.22
27.2	959	1	4	31	1	R	0.1143	Ņ	8	25	0	0	6.03	1.62
28.1	959	3	20	30	3	Ï	0.4000	N	9	30	6	50		1.02
28.2	814	3	25	15	8	Ŝ	0.6250	N	6	40	0	ő	3.60	0.67
29.1	814	4	26	14	7	S	0.6500	N	9	30	8	40	5.26	1.16
30.1	927	3	38	0	11	S	1.0000	N	10	38	ō	0		
31.1	927	3	16	19	3	Ī	0.4571	Υ	8	20	B	45	4.22	1.08
31.2	959	2	2	48	1	Ř	0.0400	N	9	15	7	50	3.70	1.30
32.1	814	3	20	15	5	I	0.5714	N	9	16	12	35	•	•
32.2	927	4	8	42	3	Ī	0.1600	Υ	10	25	18	50	8.94	1.93
33.1	959	1	5	50	2	R	0.0909	Ÿ	10	15	15	55	10.20	3.03
33.2	814	i	12	18	4	ï	0.4000	N	В	17	. 8	30	8.54	1.81
34.1	959	2	2	43	1	Ř	0.0444	N	7	20	13	45	5.70	1.35
35.1	814	4	32	0	11	S	1.0000	N	5	32	0	0	2.02	0.17
36.1	927	3	20	20	4	Ī	0.5000	Υ	10	20	11	40	3.25	0.93
36.2	959	4	2	33	1	R	0.0571	Ÿ	8	15	11	35	8.14	1.21

### ARLINGTON CLOVER -- MOTHER GENOTYPES

COLUMN NO	С	0	L	U	M	N	N	0	
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GENOTY	'PE													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
37.1	814	3	0	12	. 0	R	0.0000	Y	8	12	.0	0	2.15	0.55
37.2	927	1	3	23	1	R	0.1154	N	8	26	Ö	0	4.38	0.90
38.1	959	1	11	21	4	I	0.3438	Υ	10	32	0	0	7.82	1.75
38.2	814	1	5	35	2	R	0.1250	N	9	17	7	40	7.20	1.22
39.1	927	1	21	9	8	I	0.7000	N	9	20	0	0	14.53	3.40
39.2	959	1	2	43	1	R	0.0444	Υ	7	30	12	45	10.34	3.04
43.1	814	2	3	32	2	R	0.0857	N	5	17	3	35	5.07	1.26
43.2	927	4	7	18	3	I	0.2800	N	11	25	0	0	8.91	4.84
44.1	959	2	15	15	5	I	0.5000	N	9	30	0	0	6.78	1.15
46.1	814	3	12	18	3	I	0.4000	Υ	8	15	10	30	5.03	1.29
49.1	959	1	27	8	7	I	0.7714	N	16	11	11	35	7.70	1.83
49.2	814	3	7	28	3	Ι	0.2000	Υ	11	15	17	35	6.60	1.85
50.1	927	4	6	29	2	I	0.1714	Υ	6	35	0	0	7.51	1.04
51.1	927	1	12	18	6	I	0.4000	N	6	10	30	•	4.92	0.92
51.2	959	1	13	12	5	I	0.5200	N	12	20	0	0	7.04	2.67
52.1	814	1	23	7	9	I	0.7667	Y	9	30	0	0	7.66	2.16
52.2	927	1	21	9	7	I	0.7000	Υ	7	30	0	0	8.27	3.67
53.1	959	3	4	36	2	R	0.1000	Y	8	30	16	40	8.35	2.85
54.2	B14	3	В	37	3	1	0.1778	N	7	36	8	45	4.06	0.90
55.1	927	3	3	42	10	R	0.0667	Y	11	19	14	45	4.17	1.13
55.2	959	5	6	27	2	I	0.1818	Y	11	13	14	33	7.45	1.72
56.1	814	5	21	19	3	I	0.5250	Y	12	23	12	40	10.23	2.30
56.2	927	5	4	43	2	R	0.0851	Y	9	34	15	47	6.29	1.74
57.1	959	5	15	17	3	I	0.4688	N	9	22	11	32	3.36	1.01
57.2	814	3	3	50	2	R	0.0566	Υ	12	35	12	53	10.98	3.96
58.1	927	3	17	13	3	I	0.5667	Υ	7	15	8	30	3.28	0.93
58.2	959	6	40	0	10	S	1.0000	N	4	40	0	0	1.10	0.46
59.1	814	1	16	37	6	I	0.3019	Y	10	40	16	53	8.02	2.24
59.2	927	1	3	37	2	R	0.0750	N	9	40	0	0	8.11	2.70
62.1	927	6	9	28	3	I	0.2432	Y	6	37	0 14	0	•	•
63.1	959 959	5 5	17 9	25 44	4 3	I I	0.4048	N Y	10 7	12 39	10	30 16	•	•
64.1 64.2	814	6	22	28	7	I	0.1698 0.4400	N	5	50	0	0	•	•
65.1	927	4	2	38	1	I	0.0500		7	24	16	40	8.73	1.80
65.2	959	3	2	33	1	R	0.0571	Ÿ	6	23	9	35	5.60	1.05
66.1	814	3	2	38	1	R	0.0500	Ÿ	8	25	13	40	11.65	3.20
67.1	814	2	19	26	3	ï	0.4222	Ņ	12	15	16	45	8.92	2.29
67.2	927	5	8	47	3	ī	0.1455	Ϋ	11	22	12	33		
68.1	959	5	45	0	10	Ŝ	1.0000	N	11	45	0	0	7.17	1.92
68.2	814	5	2	31	2	R	0.0606	N	4	33	ō	ő	6.19	1.00
70.1	927	2	2	38	1	R	0.0500	Υ	10	22	12	40	8.33	2.06
71.1	927	2	2	48	1	R	0.0400	Ÿ	12	29	20	50	8.91	2.55
72.1	959	1	6	44	1	R	0.1200	N	9	20	9	50	1.95	0.74
73.1	814	6	11	39	3	Ï	0.2200	N	9	35	10	15		
74.1	814	6	32	8	9	S	0.8000	N	12	24	10	40	• 9.23	4.40
74.2	927	6	44	6	8	S	0.BB00	N	12	18	14	50	7.38	4.58
75.2	927	4	12	18	6	I	0.4000	Υ	13	30	0	0	5.25	1.25
76.1	959	3	2	33	1	Ř	0.0571	Ý	8	35	ŏ	ŏ	B.63	2.04
77.1	814	3	3	22	2	R	0.1200	Ÿ	7	25	Ö	ő	B.01	2.72
	w a 7	•	9		-		*****	•	,	~ ~			5.01	-1,2

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GENOTY	YPE -													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
78.1	959	4	2	48	1	R	0.0400	Υ	11	30	7	50	6.73	1.88
78.2	814	2	4	26	2	R	0.1333	Υ	9	30	0	0	9.11	2.20
79.1	927	1	3	33	1	R	0.0833	N	12	16	9	35	11.41	2.62
79.2	959	2	8	22	3	I	0.2667	Υ	9	18	12	30	4.31	1.06
80.1	927	3	2	28	1	R	0.0667	N	7	30	0	0	2.49	0.B3
81.1	814	2	43	2	10	S	0.9556	N	9	45	ō	ō	6.77	1.65
81.2	927	4	45	0	11	S	1.0000	N	8	25	12	45	7.72	1.73
82.1	0	2	1	31	1	R	0.0313	γ	9	20	12	32	3.95	0.93
82.2	0	1	3	47	2	R	0.0600	Ň	5	20	9	50	1.71	0.66
83.1	959	1	28	2	10	S	0.9333	N	5	30	Ö	0	4.10	0.64
84.1	959	4	6	34	3	R	0.1500	N	11	28	21	40	7.37	2.30
84.2	814	1	12	19	4	Ï	0.3871	N	8	31	0	0	3.03	0.81
86.1	814	1	2	23	1	R	0.0800	N	9	25	Ō	0	10.00	2.25
, 87.1	927	3	19	8	8	I	0.7037	N	9	22		27	5.40	1.12
87.2	959	6	12	12	5	I	0.5000	N	7	24	0	0	8.48	2.98
91.1	814	2	7	23	3	I	0.2333	N	8	24	7	30	2.42	0.75
91.2	927	6	40	0	11	S	1.0000	N	6	40	0	0	1.74	0.97
92.1	927	6	24	6	4	I	0.8000	N	12	8	11	30	8.74	4.32
93.1	959	6	25	10	5	I	0.7143	N	5	35	0	0	2.54	1.10
95.1	959	5	18	8	8	1	0.6923	N	6	26	0	0	4.44	0.88
95.2	814	4	17	4	7	I	0.8095	N	7	21	0	0		
96.1	927	5	48	2	10	S	0.9600	N	12	30	14	50	5.20	1.83
96.2	959	5	36	4	9	I	0.9000	Υ	11	40	0	0		
97.1	814	4	12	18	5	I	0.4000	N	12	30	0	0	6.55	1.45
98.1	814	5	4	31	2	R	0.1143	N	4	35	0	0		
98.2	927	1	8	22	3	I	0.2667	N	8	30	0	0	3.52	0.75
99.1	927	4	3	27	2	R	0.1000	N	13	30	. 0	0	6.95	1.90
100.1	959	4	60	0	11	S	1.0000	N	11	60	0	0	6.82	1.64
100.2	814	4	21	29	8	I	0.4200	N	8	50	0	0	3.36	0.86
101.1	927	3	11	25	3	I	0.3056	Υ	8	27	13	36	2.51	0.90
101.2	959	6	11	29	3	I	0.2750	γ	16	15	15	25		
106.1	814	1	8	27	3	I	0.2286	N	13	15	16	35	10.49	4.12
106.2	927	5	3	37	2	R	0.0750	Υ	11	29	10	11	5.42	1.77
107.1	959	1	3	37	2	R	0.0750'	N	7	20	9	40	6.39	1.86
108.1	959	6	50	10	9	S	0.8333	N	10	46	9	14		
108.2	814	5	14	16	5	1	0.4667	N	9	22	9	8	•	
110.1	814	6	29	13	4	S	0.6905	N	8	17	18	25	•	•
111.1	927	2	18	10	3	I	0.6429	N	7	28	0	0	2.02	0.68
112.1	959	6	35	0	3	S	1.0000	Ν	9	35	0	0	3.95	0.91
114.1	927	6	26	7	10	I	0.7879	N	14	33	0	0	10.87	6.71
114.2	959	4	4	28	2	R	0.1250	Y	11	13	10	32	5.68	1.42
117.1	814	3	1	39	1	R	0.0250	N	10	29	11	40	5.27	1.65
118.1	814	4	25	10	7	S	0.7143	Υ	9	35	0	0	15.75	7.51
118.2	927	3	1	29	1	R	0.0333	Y	6	19	7	30	4.68	0.92
119.1	959	2	11	24	4	I	0.3143	N	10	35	0	0	8.01	2.69
119.2	814	4	8	29	3	I	0.2162	N	13	26	16	37		
120.1	927	3	1	29	1	R	0.0333	N	5	21	· 9	30	4.1B	1.15
121.1	959	5	4	36	3	R	0.1000	Υ	13	26	13	40	5.08	2.07
123.1	927	5	2	43	1	R	0.0444	N	9	20	14	45		

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GENOTY	/PE	3	4	5	6	7	8	9	10	11	12	13	14	15
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123.2	959	5	40	5	3	S	0.8889	N	9	20	15	45		
124.1	0	6	9	23	4	R	0.2813	Y	10	22	11	10	•	•
125.1	814	5	7	38	4	I	0.1556	Y	10	28	15	45	4.15	1.10
126.1	927	1	2	43	1	R	0.0444	Υ	10	45	0	0	10.64	1.91
128.1	927	4	2	43	1	R	0.0444	N	11	15	16	45	•	•
128.2	927	4	22	16	4	I	0.5789	N	11	18	12	38		• • • •
129.1	959	2	20	15	9	I	0.5714	Y	10	35	0	0	5.69	1.15
135.1	814	6	35	5	8	5	0.8750	N	12	19	10	40	7.20	4.24
135.2	927 959	1	8 30	17 5	4 5	I S	0.3200	N Y	9 7	25 35	0	0	3.74 4.68	0.60
136.1 136.2	814	6	30	0	11	S	0.8571 1.0000	N	7	30	0	0	4.72	1.70 2.20
137.1	927	6	22	11	8	I	0.6667	N	8	33	Ö	ő	6.44	2.24
137.2	959	6	35	5	6	S	0.8750	Υ	10	15	20	40	7.4B	2.94
138.1	814	6	33	ō	10	S	1.0000	Ņ	17	18	8	33	11.99	7.91
139.1	814	6	21	11	7	I	0.6563	Y	10	32	. 0	0	5.68	1.92
139.2	927	6	2	48	2	R	0.0400	Υ	5	20		50	7.47	3.94
140.2	814	6	2	23	2	R	0.0800	N	7	25	0	0	7.83	2.61
142.2	959	2	55	25	10	S	0.6875	Υ	7	25	5	55	2.72	0.84
143.1	927	1	3	27	2	R	0.1000	N	9	30	0	0	3.38	1.04
144.1	814	1	12	23	4	I	0.3429	γ	9	26	10	35	7.29	1.92
144.2	927	2	3	47	1	R	0.0600	Y	7	30	11	50	3.94	0.85
145.1	959	2	25	20	6	S	0.5556	N	11	25	19	45	6.87	2.52
145.2	814	2	22	13	5	I	0.6286	N	12	20	13	35	3.47	1.37
154.1	959	3	8	22	3	I	0.2667	N	12	20	12	30	3.45	0.96
156.1	927	2	40	5	10	S	0.8889	N	9	22	11	45	6.15	2.05
156.2 159.1	959 959	5 5	12 12	50 18	3 3	I I	0.1935 0.4000	Y N	11 13	27 19	13 9	35	•	•
159.2	959	5	18	32	4	I	0.3600	N	9	20	10	11 30	•	•
161.1	814	5	10	35	3	Ī	0.2222	Y	7	24	13	21	5.80	2.06
163.1	927	2	3	27	2	Ř	0.1000	N	12	10	10	30	8.19	2.10
164.1	0	3	2	43	2	R	0.0444	Ϋ	8	27	10	45	2.60	
165.1	814	2	3	23	2	R	0.1154	Ÿ	10	12	16	26	8.33	1.57
165.2	927	2	6	29	3	R	0.1714	Υ	9	17	13	35	2.30	0.60
166.1	959	2	5	35	3	R	0.1250'	Υ	10	10	17	40	4.99	1.20
167.1	959	6	25	10	5	I	0.7143	N	11	11	15	35	3.78	1.47
167.2	814	6	9	30	4	I	0.2308	Y	13	14	18	39	8.11	4.41
168.1	927	6	22	23	4	I	0.4889	N	12	28	11	45	5.95	2.55
170.1	814	6	7	36	2	R	0.1628	Υ	10	16	19	27	•	•
172.1	814	6	11	26	4	I	0.2973	Υ	9	37	0	0	•	•
172.2	927	6	5	49	1	R	0.0926	N	11	15	21	39	•	•
179.1	959	4	18	17	5	I	0.5143	N	11	35	0	0		. 74
179.2 181.1	814	1	15	15	5	I	0.5000	Y	8	15	6	30	2.59	0.74
181.2	0	5 3	20 25	15 23	6 5	I I	0.5714	N	15	35	0	0	6.74	2.13
182.1	927	3 4	23 9	21	4	I	0.5208 0.3000	N N	14 9	28 21	17 8	48 30	6.92	2.21
184.1	814	3	14	16	4	Ī	0.4667	N	9	15	10	30	1.31	0.51
184.2	927	4	25	20	5	S	0.5556	N	10	25	13	45	10.91	1.66
400		_			_	_	V. 0000	14	10	2.0	13	73	10.71	1.00

2 R 0.0750 N 10 20 12 40

2 R 0.0750 Y 10 32

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4.13

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185.1 959 2

185.2 814 2

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## ARLINGTON CLOVER -- MOTHER GENOTYPES

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GENOTY	PE													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
186.2	959	4	19	11	8	I	0.6333	N	8	30	0	0		•
187.1	927	2	4	36	2	R	0.1000	Y	6	23	8	40	3.49	0.69
187.2	959	3	9	26	2	I	0.2571	Υ	10	35	0	0	11.44	2.35
188.2	0	4	18	22	5	I	0.4500	N	9	40	0	0	4.00	1.18
189.1	814	3	27	3	10	S	0.9000	N	9	30	0	0	1.69	0.50
189.2	927	5	12	38	4	I	0.2400	N	9	25	6	50	•	•
191.1	814	4	12	38	4	I	0.2400	N	10	22	18	50		•
192.1	959	4	16	19	3	I	0.4571	N	13	18	7	3 <b>5</b>		•
192.2	814	4	2	43	1	R	0.0444	N	9	22	8	45	10.31	1.49
193.1	927	5	14	26	3	I	0.3500	N	11	20	13	40	•	
196.1	927	2	15	30	4	I	0.3333	Y	9	45	0	0	5.83	0.95
196.2	959	4	4	41	2	R	0.0889	Υ	12	20	16	45	25.45	12.66
198.1	814	1	5	35	2	R	0.1250	Υ	9	22	9	40	8.98	2.28
198.2	927	1	16	19	7	I	0.4571	N	9	35	0	0	8.85	2.6B
200.1	959	4	28	12	8	S	0.7000	Υ	7	40	. 0	0	•	•
200.2	814	4	3	27	2	R	0.1000	Y	9	26	7	30	•	•
201.1	927	5	17	23	4	I	0.4250	N	10	29	15	40	•	•
201.2	959	3	3	29	2	R	0.0938	N	9	20	12	32	9.91	4.12
203.1	814	1	60	0	11	S	1.0000	N	7	60	0	0	2.43	0.83
203.2	927	5	36	14	6	S	0.7200	N	9	24	11	50	•	•
204.1	959	2	2	33	1	R	0.0571	Υ	9	35	0	0	7.33	1.79
204.2	814	6	5	30	2	R	0.1429	N	14	35	0	0	16.18	8.57
205.1	959	4	21	19	6	I	0.5250	N	9	40	0	0	1.86	0.56
206.1	814	2	9	26	3	I	0.2571	N	8	26 '	8	35	6.48	1.25
207.2	0	5	4	26	2	R	0.1333	Υ	8	30.	0	0	6.77	1.94
208.1	927	2	4	29	2	R	0.1212	Υ	6	25		33	3.20	1.14
208.2	959	2	8	26	2	I	0.2353	Υ	9	23	14	34	6.55	1.63
209.1	927	2	7	18	3	I	0.2800	N	6	25	0	0	2.40	0.65

#### FLOREX CLOVER -- MOTHER GENOTYPES

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GENOTY	/PE													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
210.1	814	6	50	0	11	S	1.0000	N	7	50	0	0		11.73
210.2	927	3	20	16	8	1	0.5556	Y	6	36	0	0	2.40	0.93
212.2	814	4	17	19	3	I	0.4722	Υ	10	25	8	36	•	•
213.1	927	3	22	13	7	I	0.6286	Y	7	35	0	0	2.85	0.B5
213.2	959	3	27	3	9	S	0.9000	N	6	30	0	0	4.57	1.15
214.1	814	4	16	21	4	I	0.4324	Υ	11	12	15	37	•	•
215.1	927	4	3	42	2	R	0.0667	N	11	20	18	45	•	•
216.1	959	4	2	43	1	R	0.0444	N	11	10	26	45		. •
218.1	814	2	16	16	3	I	0.5000	Y	11	12	7	32	7.50	1.65
218.2	927	1	10	20	3	I	0.3333	Y	11	30	0	. 0	4.38	0.95
220.1	959	1.	10	15	3	I	0.4000	N	9	25	0	0	4.12	0.77
220.2	814	1	22	8	4	I	0.7333	N	7	30	0	0	2.60	0.65
221.1	927	3	0	30	0	R	0.0000	Y	12	19	10	30	8.14	2.79
, 221.2	959	3	2	28	1	R	0.0667	Y	14	18	11	30	11.04	3.57
223.1	814	2	14	16	4	I	0.4667	N	10	30	0	0		1 22
224.1	814	1	23	22	5	I	0.5111	N	8	24	11	45	4.16	1.22
225.1 225.2	959 814	2	· 4	32 15	1 5	R	0.1111	Y Y	7 9	22 21	12	36 30	3.40 1.72	0.B2 0.60
226.1	927	1	7	19	3	I I	0.2692	N	6	26	0	0	5.60	1.71
227.1	814	2	11	9	3 7	I	0.5500	N	7	20	0	0	1.83	0.64
227.1	927	1	12	18	4	I	0.4000	Y	12	30	0	0	3.86	1.34
228.1	927	1.	7	28	2	I	0.2000	Y	5	35	ŏ	0	2.42	0.56
229.1	0	5	4	26	2	R	0.1333	N	10	19	14	30	4.35	1.57
229.2	Ö	2	0	66	0	R	V.1555	N	9	17	0	49	5.02	1.96
230.1	959	1	13	13	3	ï	0.5000	N	8	26	ŏ	0	2.82	0.50
231.1	0	4	6	27	2	Ī	0.1818	N	12	8	15	33	7.45	2.08
235.1	959	1	40	0	11	Ŝ	1.0000	N	5	40	0	0	1.77	0.48
235.2	814	2	27	13	8	S	0.6750	N	8	20	8	40	1.91	0.69
236.1	927	1	2	28	1	R	0.0667	Υ	7	30	ō	ő	3.84	1.09
236.2	959	4	8	32	3	Ï	0.2000	N	10	10	11	40	7.55	1.59
237.1	814	4	12	18	7	Ī	0.4000	N	9	30	0	0	3.55	1.44
238.1	927	4	4	38	2	R	0.0952	Υ	11	31	18	42	7.19	1.78
239.2	959	6	2	28	1	R	0.0667	Υ	11	17	10	30	3.72	0.94
240.1	814	4	31	11	5 `	S	0.7381	N	10	22	19	42	8.26	1.66
240.2	927	4	3	33	2	Ŕ	0.0833	Υ	9	11	19	36	4.49	1.00
241.1	814	5	9	26	3	I	0.2571	Y	13	20	17	15	11.77	3.28
242.1	927	2 :	2	28	1	R	0.0667	N	8	30	0	0	7.94	2.14
243.1	959	4	9	26	3	I	0.2571	Υ	11	10	18	35	9.44	2.61
243.2	814	2	8	17	3	I	0.3200	Y	9	15	•	25	4.57	0.91
244.1	959	2	20	23	5	I	0.4651	N	9	19	13	43	10.29	1.81
245.1	927	2	39	0	10	S	1.0000	N	12	30	•	39	3.39	1.74
245.2	959	1	5	23	0	R	0.1786	Y	11	28	0	0	1.62	0.62
246.1	0	2	0	35	0	R	0.0000	Υ	10	15	9	35	2.09	0.75
247.1	814	4	13	24	5	I	0.3514	N	7	37	0	0	4.25	1.06
249.1	927	1	6	23	2	I	0.2069	N	9	29	0	0	2.27	0.65
250.1	0	6	3	32	1	R	0.0857	N	6	35	0	0	4.95	0.58
252.1	0	6	2	43	1	R	0.0444	N	9	9	21	36	6.64	1.93
253.1	814	.4	18	16	4	I	0.5294	Y	8	34	0	0	2.98	0.65
253.2	927	4	7	38	2	I	0.1556	Υ	10	23	7	45	4.72	0.90

r	п	1	11	М	N	N	п	
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GENOTY	/PE													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
254.1	959	4.	7	33	3	I	0.1750	N	12	31	14	40	11.23	2.60
254.2	814	4	3	52	2	R	0.0545	N	10	25	13	55	9.32	2.40
255.1	927	4	11	29	3	I	0.2750	γ	11	15	14	40	7.23	1.72
256.1	0	5	1	34	1	R	0.0286	Ÿ	10	13	6	35	4.31	1.01
256.2	ō	1	14	13	4	I	0.5185	N	5	27	ō	0	1.26	0.41
257.1	814	3	21	9	8	Ī	0.7000	N	7	30	Ō	0	11.27	4.18
257.2	927	1	5	30	2	Ř	0.1429	N	11	35	0	0	7.60	3.30
258.1	959	1	18	12	5	I	0.6000	Υ	15	30	0	0	3.48	1.26
258.2	814	1	30	5	9	S	0.8571	N	9	35	0	0	1.76	1.00
259.1	927	1	6	27	2	I	0.1818	Υ	9	25	10	33	5.30	1.42
259.2	959	5	4	56	2	R	0.0667	γ	8	15	13	60	5.78	1.79
260.2	927	4	11	34	4	I	0.2444	Υ	13	10	18	45	5.60	1.84
261.1	959	4	2	53	1	R	0.0364	Υ	10	15	15	55	6.39	1.96
261.2	814	2	11	22	3	I	0.3333	Υ	8	10	22	33	2.35	0.75
262.1	927	3	15	15	4	1	0.5000	N	7	30	0	0	3.80	0.60
263.1	814	6	26	7	4	5	0.7879	N	7	17	11	33	B.03	1.94
263.2	927	3	9	25	3	I	0.2647	N	6	34	0	0	6.24	1.56
264.1	959	3	2	28	1	R	0.0667	Υ	13	30	0	0	7.51	1.26
266.1	959	3	11	19	3	1	0.3667	N	8	16	6	30	3.85	0.50
266.2	814	3	11	19	4	I	0.3667	N	7	30	0	0	4.63	0.68
267.1	927	3	3	47	1	R	0.0600	Υ	9	20	12	50	7.29	0.77
267.2	959	2	3	32	1	R	0.0857	Υ	10	25		35	4.68	1.56
268.1	814	3	28	5	10	S	0.8485	N	7	33	0	0	8.61	1.50
269.1	814	3	3	32	2	R	0.0857	Υ	8	25	14	35		•
269.2	927	1	3	29	1	R	0.0938	Υ	7	32	0	0	4.90	1.08
270.1	959	3	1	31	1	R	0.0313	N	10	12	17	32	4.97	1.97
270.2	814	3	2	43	1	R	0.0444	Y	11	28	18	45	7.19	3.35
271.1	927	1	3	33	1	R	0.0833	N	8	36	0	0	5.75	1.75
271.2	959	2	3	26	1	R	0.1034	Υ	10	16	7	29	2.79	0.90
272.1	927	6	3	27	2	R	0.1000	N	8	30	0	0	•	•
273.1	0	5	2	38	1	R	0.0500	Υ	8	22	12	40	5.17	1.00
273.2	0	1	0	30	0	R	0.0000	N	12	30	0	0	6.39	2.02
274.1	0	2	10	35	4	I	0.2222	Y	5	45	0	0	3.61	0.82
274.2	0	1	3	23	1	R	0.1154	N	6	26	0	0	5.84	1.20
275.1	814	3	26	9	9	S	0.7429	N	8	25	11	35	•	•
275.2	927	2	11	34	4	I	0.2444	N	8	45	0	0	4.74	1.18
277.1	959	2	0	30	0	R	0.0000	N	7	30	0	0	3.22	0.76
277.2	814	2	4	21	2	R	0.1600	γ	10	25	0	0	4.69	1.26
278.1	927	4	16	19	6	I	0.4571	Υ	9	35	0	0	16.09	2.92
278.2	959	4	32	23	6	S	0.5818	Y	12	33	16	55	8.47	2.17
279.1	814	5	50	0	11	S	1.0000	N	4	50	0	0	2.46	0.15
279.2	927	4	35	0	11	S	1.0000	N	4	35	0	0		
280.1	959	4	5	22	3	R	0.1852	Y	9	14	9	27	7.63	1.20
281.1	814	1	30	10	10	S	0.7500	N	7	40	0	0	5.76	0.56
282.1	959	3	17	18	4	I	0.4857	N	8	14	3	35	5.17	1.03
282.2	814	4	13	12	5	I	0.5200	N	9	25	0	0	6.53	0.57
283.2	959	5	27	3	10	S	0.9000	γ	9	23	7	7	7.49	0.51
284.1	927	5	6	44	3	I	0.1200	Y	4	50	0	0	•	•
285.1	959	6	11	36	2	I	0.2340	Y	9	26	7	21	•	•

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GENOTY	YPE													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
286.1	814	3	22	13	7	I	0.6286	N	11	35	0	0	3.14	1.01
286.2	927	2	12	12	4	I	0.5000	N	8	24	0	0	1.83	0.34
288.1	927	2	6	24	3	I	0.2000	N	5	30	0	0	1.58	0.59
289.1	959	3	21	14	3	I	0.6000	N	12	17	8	35	9.43	4.20
289.2	814	2	22	0	11	I	1.0000	N	3	22	0	0	0.15	0.09
290.1	959	2	7	33	2	I	0.1750	N	6	40	0	0	1.63	0.56
293.1	927	2	7	23	2	I	0.2333	Υ	11	30	0	0	3.74	0.97
293.2	959	2	0	35	0	R	0.0000	N	9	35	0	0	3.13	0.74
294.1	814	3	30	0	3	S	1.0000	N	7	30	0	0	2.74	0.62
295.1	814	1	4	21	1	R	0.1600	N	9	25	6	•	3.73	0.87
296.1	959	4	17	23	4	I	0.4250	Υ	9	25	14	15	•	•
296.2	814	4	2	43	1	R	0.0444	N	8	32	14	45	8.90	1.98
297.1	927	3	21	19	6	I	0.5250	Υ	10	19	5	40	7.67	1.21
, 297.2	959	4	11	29	3	I	0.2750	N	7	40	0	0	5.16	0.97
298.1	814	1	12	20	3	I	0.3750	N	10	32	2	•	7.65	4.21
298.2	927	3	1	61	1	R	0.0161	N	13	22	13	62	6.30	2.71
300.1	814	6	9	22	4	I	0.2903	Y	16	21	19	33	10.66	3.1 <b>5</b>
300.2	814	3	10	20	3	I	0.3333	N	11	18	14	30	2.77	1.11
302.1	927	2	11	29	3	I	0.2750	Υ	8	24	13	40	5.36	1.74
302.2	959	1	4	28	1	R	0.1250	Y	7	18	9	32	6.26	1.49
303.1	0	1	2	28	1	R	0.0625	N	10	30	0	0	2.41	0.64
305.2	927	6	4	47	2	R	0.0784	Y	11	16	19	51	10.32	3.13
306.1	959	5	8	47	3	I	0.1455	Υ	10	36	18	19	•	•
306.2	814	5	15	35	4	I	0.3000	N	11	20	24	30	12.16	3.84
308.1	927	5	26	19	7	S	0.5778	Υ	11	24	12	21	6.08	1.74
308.2	959	5	15	20	3	I	0.4286	N	9	12	7	23	4.57	1.00
309.1	927	5	32	8	8	S	0.8000	N	14	21	22	40	6.04	2.18
310.1	814	5	25	15	5	S	0.6250	N	13	12	10	40	4.93	1.41
310.2 311.1	927 959	5 5	6	44 22	2 4	I	0.1200	Y	7	15	14	35	2.69	1.06
311.2	814	6	23 35	10	10	I S	0.5111	N	12 8	25 45	15	20	5.87	0.97
312.1	927	4	16	14	5	I	0.7778 0.7273	N N	9	22	0 8	0 8	5.70	1.35
312.2	959	1	28	2	9	5	0.7273	Y	7	30	0	0	7.83 7.30	1.42 2.78
313.1	959	2	0	32	ó	R	0.0000		12	32	ŏ	ŏ	6.68	1.64
314.1	814	1	2	31	1	R	0.0606	N	6	33	ő	ŏ	2.22	0.52
314.2	927	2	13	17	5	Ï	0.4333	N	8	30	ō	ŏ	1.94	0.95
315.1	814	6	35	0	11	S	1.0000	N	5	35	ő	Õ	12.03	3.89
316.1	959	5	32	13	5	S	0.7111	N	9	20	8	25	9.27	2.31
316.2	814	5	20	30	4	Ī	0.4000	γ	9	25	6	25	7.50	1.42
317.2	959	3	5	35	2	R	0.1250	Ÿ	9	22	11	40	4.44	1.22
318.1	814	2	7	27	2	Ï	0.2059	N	6	29	8	34	12.87	1.55
318.2	927	1	11	19	3	Ī	0.3667	N	6	30	ō	0	2.58	0.96
319.1	959	3	10	20	4	Ī	0.3333	N	10	30	ŏ	ŏ	4.79	1.22
319.2	814	1	40	0	10	S	1.0000	N	6	40	ŏ	ŏ	3.80	0.99
320.1	959	2	9	31	3	Ī	0.2250	Y	В	40	ő	ŏ	2.54	0.72
320.2	959	2	5	25	2	R	0.1667	Ÿ	5	30	ŏ	ŏ	4.74	1.29
321.1	814	1	15	15	4	Ï	0.5000	N	5	30	ŏ	Ö	2.83	0.49
322.1	927	6	14	21	6	Ī	0.4000	N	9	35	ŏ	ŏ	7.15	1.71
323.1	959	6	13	14	4	Ī	0.4815	N	7	27	Ŏ	ŏ		•

r	n	1	11	М	M	N	п	
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GENOT	YPE -													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
327.1	927	6	40	0	11	S	1.0000	N	3	40	0	0	3.15	0.87
330.1	959	6	30	5	9	S	0.8571	N	14	19	14	16		•
331.1	959	1	1	14	1	R	0.0625	N	9	15	0	0	2.77	0.66
331.2	814	1	18	22	3	I	0.4500	Υ	9	22	16	40	3.93	0.92
333.1	814	1	16	14	8	1	0.5333	N	9	30	0	0	5.43	1.06
334.1	927	3	2	43	1	R	0.0444	Υ	9	22	20	45	8.35	1.91
334.2	959	4	6	34	2	I	0.1500	N	10	15	7	40	7.83	1.43
335.1	927	3	6	54	2	I	0.1000	Υ	10	25	14	60	•	
336.1	959	3	4	36	2	R	0.1000	N	9	40	0	0	•	•
337.1	814	4	21	29	5	I	0.4200	Υ	7	40	13	50	2.27	0.94
339.1	814	5	8	29	3	1	0.2162	N	9	37	0	0		
339.2	927	2	20	15	7	I	0.5714	N	7	35	0	0	0.97	0.35
340.1	959	5	27	33	4	S	0.4500	Υ	9	22	14	38	9.82	2.17
341.1	814	5	2	53	1	R	0.0364	Y	9	40	10	15	2.35	0.96
342.1	927	2	16	19	5	I	0.4571	N	13	19		35	2.50	0.97
343.1	959	2	30	0	11	S	1.0000	N	6	30	0	0	0.74	0.30
345.1	959	2	9	21	2	I	0.3000	Υ	В	30	0	0	4.56	1.24
345.2	814	3	11	39	3	I	0.2200	Υ	7	50	0	0	3.74	1.02
346.2	959	4	11	19	3	I	0.3667	Y	5	30	0	0	3.07	0.65
347.1	814	3	4	28	2	R	0.1250	Υ	14	20	17	32	6.56	1.84
350.1	814	5	16	22	4	I	0.4211	N	10	18	16	38	6.33	1.90
350.2	927	5	0	42	0	R	0.0000	N	8	13	14	29		
351.1	959	6	50	0	10	S	1.0000	N	9	40	12	50	12.73	3.26
353.1	959	6	12	38	3	I	0.2400	N	8	35	15	50	13.16	2.40
354.1	927	6	35	0	10	S	1.0000	N	8	35	0	0	5.35	1.74
354.2	959	5	1	34	1	R	0.0286	Υ	11	19	21	16	•	
355.1	927	5	9	31	3	1	0.2250	Υ	10	15	18	25	6.93	2.11
357.1	959	5	3	43	2	R	0.0652	N	10	25	8	46	2.86	0.42
357.2	814	5	23	26	5	I	0.4694	N	8	27	9	49	4.60	0.99
358.2	927	6	7	33	2	I	0.1750	Υ	10	20	13	40	12.87	2.17
359.2	959	6	5	35	2	R	0.1250	N	6	40	0	0	8.12	1.16
360.1	814	5	17	13	8	I	0.5667	N	13	30	0	0	9.63	3.03
360.2	927	5	30	5	9	S	0.8571	N	10	20	14	15	5.60	1.04
362.1	959	6	3	30	1	R	0.0909	, N	9	33	0	0	3.29	1.02
362.2	814	6	24	6	6	1	0.8000	Υ	14	13	14	30	12.23	5.52
364.1	814	5	6	44	2	I	0.1200	Υ	8	20	7	30	3.30	0.49
364.2	814	6	27	9	7	S	0.7500	N	9	24	8	36	3.81	1.27
366.1	927	5	28	12	7	S	0.7000	N	13	19	13	21		
366.2	959	5	12	28	3	I	0.3000	N	9	21	19	19		
367.2	927	2	9	18	3	I	0.3462	N	7	27	0	0	1.56	0.42
370.1	959	3	1	29	1	R	0.0333	N	8	12	14	30	8.75	1.89
371.1	927	5	45	0	10	S	1.0000	N	4	45	0	0	10.38	2.07
372.1	927	5	14	26	3	I	0.3500	N	10	23	15	40	7.63	2.11
373.1	814	5	19	19	5	I	0.7917	N	9	19	8	38	4.27	1.03
374.2	927	5	1	44	1	R	0.0222	N	5	25	11	20	•	
375.2	959	5	4	39	2	R	0.0930	Y	8	13	20	30	6.12	1.48
377.1	814	6	30	0	11	S	0.5000	N	ō	30	0	0	2.30	0.3B
378.2	814	6	8	34	4	Ī	0.1905	N	9	16	16	42	5.31	1.48
386.1	814	1	40	Ō	10	S	1.0000	N	5	40	0	0	2.05	0.87

### FLOREX CLOVER -- MOTHER GENOTYPES

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GENOTY	/PF													
#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
386.2	927	3	23	17	8	I	0.5750	γ	6	15	5	40	2.24	0.65
387.1	814	2	25	15	7	S	0.6250	N	9	25	8	40	1.38	0.54
388.1	927	3	5	39	2	R	0.1136	N	9	19	8	44	2.99	0.BB
389.1	959	1	28	2	9	S	0.9333	Υ	4	30	0	0	1.71	0.49
391.1	959	5	35	10	6	S	0.7778	N	9	10	25	45	4.29	1.17
391.2	814	2	6	24	3	I	0.2000	N	11	15	7	30	4.38	1.09
392.1	927	4	7	33	3	I	0.1750	Y	12	18	12	40	7.93	2.24
393.1	814	4	23	2	10	Ι	0.9200	N	6	25	0	0	2.55	0.53
393.2	927	4	12	28	4	I	0.3000	N	10	24	8	40	3.17	0.99
394.1	927	4	9	38	4	I	0.1915	Υ	13	20	7	47	5.72	1.35
395.1	959	4	3	42	2	R	0.0667	Y	11	25	9	45	5.69	1.44
395.2	814	4	19	6	7	I	0.7600	N	7	25	0	0	5.28	1.22
396.1	0	5	1	54	1	R	0.0182	Υ	12	25	15	30	5.53	1.52
397.2	959	6	11	24	3	I	0.3143	Υ	10	14	16	35	10.21	3.32
398.2	959	1	28	1	8	S	0.9655	Υ	10	29	0	0	5.58	2.19
399.1	0	4	2	28	2	R	0.0667	N	9	10	5	30	0.81	0.40
400.1	0	3	0	22	0	R	0.0000	Υ	8	22	14	•	3.68	0.59
400.2	0	3	0	12	0	R	0.0000	Υ	11	12	•	35	3.71	0.91
401.1	0	5	3	37	2	R	0.0750	Υ	9	27	6	40	7.72	1.75
401.2	0	1	0	42	0	R	0.0000	Y	7	42	0	0	4.23	1.44
402.1	0	4	0	20	0	R	0.0000	Υ	15	20	. 0	0	7.29	1.51
404.1	927	2	20	15	7	I	0.5714	N	10	35	0	0	4.95	1.24
404.2	959	3	3	27	2	R	0.1000	Υ	12	30	0	0	8.11	1.84
407.1	814	3	20	20	5	1	0.5000	N	4	20	3	40	2.79	0.95
410.1	927	5	5	35	2	R	0.1250	N	9	32	6	8	•	•
411.1	959	6	17	33	5	I	0.3400	N	8	50	0	0	4.69	1.19
411.2	959	6	10	30	3	I	0.2500	N	9	18	10	40	8.32	2.07
413.1	959	1	24	2	10	I	0.9231	N	6	26	0	0	4.13	0.85
414.1	927	1	0	25	0	R	0.0000	N	10	25	0	0	6.04	2.18
414.2	959	1	3	20	1	R	0.1304	N	12	20	6	25	8.45	2.95

### VII.2 APPENDIX 2

# RED CLOVER F1 PLANTS RESPONSE TO FUSARIUM RACE 814

COLUMN	VARIABLE	DESCRIPTION
2	PLANT #	PLANT REPLICATE WITHIN THE CROSS
3	FUNGUS	FUSARIUM ISOLATE NUMBER (000 is control)
4	IL	ROOT INFECTION LENGTH, in mm
5	10	DISTANCE OF UNINFECTED TISSUES FROM
		INFECTION SITE TO CROWN, in mm
6	НВ	HORSFALL-BARRATT SCALE
7	IB	INTERNAL BREAKDOWN (Y or N)
8	IBW	INTERNAL BREAKDOWN WIDTH, in mm
9	IBD	INFECTION OF INTERNAL BREAKDOWN (Y or N)
, 10	CW	CROWN WIDTH, in mm
11	TAPLEN	TAFROOT LENGTH, in mm
12	TPW	TOTAL PLANT WEIGHT, in grams
13	TRW	TAPROOT WEIGHT, in grams

### CROSSES -- ARLINGTON CLOVER

_			 			_	
	n	1	 m	NI	N	11	
			 1.1	I.A	14		

								CO	LU	M N	١	10.			
	CR	# #		2	3	4	5	6	7	8	9	10	11	12	13
	1	χ	6	1	814	7	23	3	N		N	14	30	2.70	1.27
	1	X	6	2	814	16	24	4	N	ì	N	10	40	3.23	1.72
	1	X	6	3	814	13	27	6	N		N	10	45	2.80	1.55
	1	X	6	4	814	7	23	3	Υ	9	N	12	30		1.52
	1	X	6	5	814	20	20	4	γ	1	N	10	40	2.71	1.33
	1	X	6	6	814	22	18	5	N		N	11	40	2.38	0.90
	1	X	6	7	814	8	32	4	Υ	4	Y	13	40	3.33	1.32
		X	6	В	814	27	15	7	N		N	9	42	2.56	1.01
		X	6	9	814	11	29	4	Υ	5	Y	12	40	5.35	2.99
		X	6	10	814	17	25	5	Y	5	Y	14	42	5.46	2.68
		X	6	11	814	19	26	6	N	•	N	11	45	4.52	1.86
		X	6	12	814	16	24	5	N	•	N	11	40	4.60	1.75
		X	6	13	814	26	19	9	N	:	N	8	35	3.14	1.04
•		X	6	14	814	10	25	4	Y	3	N	12	35	3.92	1.29
		X	6	15	814	19	14	5	N	•	N	14	35	4.76	2.17
		X X	6	16 17	814	11	29	4	N	•	N	7	40	3.93	0.90
		X	6	18	814 814	17 12	18 28	6 4	N Y	4	N Y	8 9	35 40	4.23 3.40	1.23
		X	6	19	814	19	21	6	N		N	11	40	2.75	1.43
		X	6	20	814	30	6	9	N	•	N	10	36	2.78	0.85
		X	6	21	814	20	15	5	Υ	3	Y	9	35	2.11	1.00
		X	6	22	814	4	26	3	Ϋ́	2	N	10	30	3.75	1.11
		X	6	23	814	7	30	3	Ϋ́	2	N	14	37	5.10	2.07
		X	6	24	814	11	19	4	N	-	N	10	30	3.52	0.93
		X	6	25	814	21	16	6	N		N	10	37	3.82	1.80
		X	6	26	B14	17	23	4	γ	4	Υ	9	40	2.27	0.90
		X	6	27	814	12	18	4	Υ	3	N	13	30	3.03	1.52
	1	X	6	28	814	17	23	5	Y	5	Υ	11	40	3.63	2.10
	1	X	6	29	814	10	25	4	N		N	8	<b>35</b>	1.61	0.48
			6	30	814	10	30	3	N	9	N	9	40	4.71	1.14
			6	31	814	20	20	5	N	• .	N	10	40	4.15	1.09
			6	32	B14	17	18	5	N	•	N	7	35	2.77	0.99
			6	33	814	5	45	2	Y	2	Υ	10	50	5.20	2.57
			6	34	814	22	18	4	N	٠.	N	6	40	3.81	1.07
			6	35	814	25	15	8	N	•	N	6	40	2.44	0.82
			6 6	36 37	814 814	1 1 4	29 31	4	N	•	N	9	40	3.24	1.07
			6	38	814	8	32	2 3	N N	•	N N	6 9	35 40	2.15 3.88	0.70 0.9B
			6	39	814	18	22	5	N	•	N	9	40	3.95	1.12
			6	40	814	4	36	3	N	:	N	10	40	4.00	1.35
			6	41	000	3	32	1	N	•	N	12	35	3.16	1.12
			6	42	000	1	39	ō	Y	i	γ	6	40	3.12	0.74
	1)		6	43	000	2	38	ŏ	N		N	9	40	2.55	1.04
	6)		1	1	814	19	28	4	N	÷	N	9	47	3.26	1.86
	6)		1	2	814	18	22	4	N	:	N	9	40	3.15	1.24
	6 )		1	3	B14	5	35	2	Υ	2	N	9	40	2.88	1.27
	6 X		1	4	814	7	29	3	N		N	b	36	2.76	0.56
	6 X		1	5	814	25	15	7	N		N	9	40	2.52	0.99
	6 X		1	6	814	13	27	4	N		N	5	50	3.49	0.84

### CROSSES -- ARLINGTON CLOVER

-	r	n	11	М	N	N	U	

								CO	LU	M N	N	υ.			
	CF	ROS!	5	2	3	4	5	6	7	8	 9 	10	11	12	13
•	6	X	1	7	814	11	29	3	Υ	1	N	11	40	2.27	0.71
	6	Ŷ	i	8	814	29	16	4	N		N	6	45	1.47	0.96
	6	x	1	9	814	25	25	4	Y	2	N	10	50	4.50	2.37
	6	Ŷ	i	10	814	26	11	8	Ÿ	2	N	11	37	3.09	1.31
	6	x	1	11	B14	6	29	2	N	-	N	10	35	3.17	1.22
	6	X	1	12	814	1	45	ō	Ϋ́	1	Υ	8	45	3.05	0.77
	6	X	1	13	814	13	27	5	N	•	N	8	40	2.48	0.82
	6	χ	i	14	814	4	31	2	Y	2	N	10	35	2.95	1.01
	6	X	1	15	814	3	33	2	N		N	6	36	3.15	1.07
		X	1	16	814	17	23	5	N	:	N	7	40	1.33	0.82
		X	1	17	814	12	2B	3	Ÿ	4	γ	11	40	4.15	1.79
	6		1	18	B14	3	47	1	N	7	N	9	50	3.84	1.81
	6	X X	1	19	814	12	28	4	N	•	N	9	40	1.68	0.65
	6				814	14	26	3	Y	1	N	9	40	2.33	0.93
	6	X	1	20 21	814	12	28	3	N	•	N	3	40	2.18	0.75
	6	X X		22	814	12	20	٥	14	•	"		70	2.10	0.,,
	6	X	1	23	814	2	38	1	Υ	1	N	7	40	2.40	0.75
	6	X	1	24	814	2	36	•	•	•	14	,	10	2	V., .
	6	χ	1	25	814	45	0	11	N		N	7	45	0.61	0.61
	6			26	814	14	21	6	N	•	N	10	35	3.04	1.18
	6	X	1	27	814	9	21	5	Y	3	Y	12	30	3.51	1.47
	6	X	1 1	28	814	3	37	2	N		N	10	40	3.33	1.0B
	6	X X	1	29	814	6	44	2	N	:	N	6	50	2.58	1.16
	6		1	30	814	12	33	3	N	•	N	11	45	2.98	1.47
	6	X	1	31		13	2 <b>9</b>	5	N		N	9	42	3.04	1.32
	6	X		32	814	14	21	4	N	•	N	10	35	2.81	0.92
	6	X	1		814	2	42	1	Υ	i	Y	11	44	4.80	1.67
	6	X	1	33 34	814	20	10	5	N	•	N	10	30	3.90	1.87
	6	X	1	35	814	14	30	3	N	•	N	11	44	3.26	1.73
	6	X	1	36	814 814	24	31	7	Υ	• 4	Y	10	55	3.74	1.47
	6	X	1	37	814	2	33	2	Ÿ	2	N	13	35	3.03	1.69
	6 6	X	1	38	814	7	28	3	Ņ	_	N	7	35	3.85	1.52
	6	X	1	39	814	29	8	8	N	•	N	10	37	3.62	1.23
	6	Ŷ	1	40	814	17	23	4	N	`.	N	8	40	1.55	0.90
	6	x	1	41	000	1	34	2	Ÿ	1	N	13	35	4.78	1.94
	6	x	i	42	000	11	28	4	Ň	-	N	12	39	3.02	1.80
	6	X	1	43	000	1	49	0	N	·	N	11	50	3.76	1.87
	6	χ	1	44	000	i	36	i	N	•	N	10	37	3.45	1.38
	6	X	1	45	000	i	30	0	N		N	10	30	3.98	1.83
	6	X	28	1	814	20	11	8	N		N	9	31	3.58	1.BO
	6	X	28	2	814	2	33	0	Y	3	N	12	35	4.15	1.92
	6	x	28	3	B14	4	36	3	N		N	8	40		
	6	X	28	4	814	13	22	5	N	:	N	11	35	·	
	6	χ	28	5	814	24	11	10	Y	2	N	11	3 <b>5</b>		
	6	χ	28	6	814	11	24	4	Ņ		N	8	35		
	6	χ	28	7	814	7	33	3	N	•	N	9	40		
	6	X	28	8	814	14	23	5	Y	3	N	10	40		
	6	Ŷ	28	9	814	9	26	4	N		N	6	35		
		X	28	10	814	6	29	3	N	:	N	8	35		
					U 1 T		- /	_				_			

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										м	ki	M	n			
	r r	one	e					C 0					0.			
	LI	10S #	5	2	3	4	5	6	7		8	9	10	11	12	13
•	6	x X	28	11	814	12	18	5	N			N	10	30		
	6	X	28	12	814	7	28	3	N			N	12	35		
	6		28	13	814	6	26	3	Υ		2	N	11	32		
		X	28	14	814	25	10	10	N			N	9	35		
		X	28	15	814	1	31	0	γ		2	Υ	11	32		
		X	28	16	000	1	29	0	Υ		2	Υ	12	30		
	6		28	17	000	1	34	0	N			N	8	35		
	6		28	18	000	1	32	0	N			N	9	33		
	28		6	1	814	15	20	6	Y		2	N	11	35		
		X	6	2	814	8	27	4	N			N	7	35		
	28		6	3	814	26	6	10	γ		2	N	12	32		
	28	X	6	4	814	2	31	1	N			N	10	33		
	28		6	5		7	23	4	N			N	8	30		
٨	28		6	6	B14	6	29	3	N			N	10	35		
•	28		6	7	814	9	24	5	N			N	8	33		
	28		6	8	814	8	25	5	Y		1	N	11	33		
	28		6	9	814	9	26	5	N			N	10	35		
	28	X	6	10	814	9	26	5	N		•	N	10	35		
	28	X	6	11	814	6	29	4	N		•	N	8	35		
	28	X	6	12	814	10	22	7	γ		2	Y	12	32		
	28	X	6	13	814	21	11	9	Υ		1	Υ	11	32		
	28	X	6	14	814	7	25	4	N		•	N	9	32		
	28	X	6	15	B14	3	29	2	Υ		1	N	10	32		
	28	X	6	16	814	18	12	8	Υ		2	N	11	30		
	28	X	6	17	814	11	19	6	Υ		1	N	10	30		
	28	X	6	18	814	14	21	7	N		•	N	9	36		
	28	· X	6	19	814	2	35	1	N		•	N	10	35		
	28	X	6	20	B14	7	25	4			2	N	10	32	*	
	28		6	21	000	2	38	1	N		•	N	9	40		
	28		6	22	000	1	29	0	N		•	N	11	30		
	28	X	6	23	000	1	34	0	N		•	N	9	35		
	28	X	6	24	000	2	38	1	N		•	N	10	40	2 00	A 74
	20	X	29	1	814	21	9	6	N		•	N	9	30 70	2.00	0.74
	20	X	29	2	814	7	23	3	N		•	N	B 7	30 3 <b>5</b>	2.09 1.91	0.61
	20	X	29	3	814	6	29	3	N		•	N		30	1.82	1.01
	20	X	29	4	814	11	19	4	N N		•	N N	10 10	30	2.70	1.53
	20	X	29	5	814	19	11 13	6 5	N		•	N	9	30	2.25	1.17
	20	X	29	6	814	17 3	27	3	N		•	N	7	30	2.34	0.58
	20	X	29	7	814 814	18	17	4	N		٠	N	4	35	1.73	0.63
	20 20	X	29 29	8 9	814	10	25	3	N		•	N	6	35	2.93	1.14
		X	29	10	814	10	24	3	N			N	9	34	2.39	0.97
		X	29 29	11	814	35	35	10	N			N	7	35	1.78	0.52
	20	χ	29 29	12	B14	18	18	4	N		•	N	. 9	36	3.14	1.65
	20	X	29 29	13	814	11	19	4	N			N	10	30	2.73	1.29
	20	X	27 29	14	814	24	10	8	N		:	N	6	34	3.76	1.23
		X	27	15	814	14	21	3	Y		1	N	10	35	2.63	1.46
		X	29	16	814	1B	17	4	Y		i	N	10	35	2.89	1.23
	20		29	17	814	21	14	6	N			N	9	35	2.43	0.94
	20	^	2. /	.,	017	~ 1	• 1	J	.,		•	••	,		· <del>-</del>	

~	~			N	м	0	
			m	T/I	- 14	11	
	1.3	u		13		u	

	C	ROS	20							 					
		#		2	3	4	5	6	7	8	9	10	11	12	13
	20		29	18	814	14	20	4	N		N	9	34	2.85	1.35
	20		29	19	814	16	14	4	N	•	N	9	30	2.23	1.08
	20		29	20	814	15	15	4	N	•	N	10	30	3.21	1.63
	20		29	21	814	10	27	3	N	•	N	8	37	2.36	1.05
	20		29	22	814	11	22	3	N	•	N	9	33	2.84	0.98
	20		29	23	814	31	4	10	N	•	N	6	35	1.25	0.58
	20		29	24	814	19	13	4	Y	1	N	10	32	2.61	1.18
	20		29	25	814	22	13	5	N	•	N	5	35	1.84	0.65
	20		29	26	814	17	18	4	N	•	N	9	35	2.58	0.99
	20		29	27	814	14	21	4	N	•	N	9	35	1.94	0.79
	20		29	28	814	19	16	5	Y	2	N	9	35	3.30	1.58
	20		29 29	29 30	814	17 10	18 25	4	N N	•	N N	8 8	35 35	1.90	1.09
	20 20		29	31	814 814	21	11	3 3	Y	1	N	9	32	1.59	1.05
•	20		29	32	814	16	19	3	N		N	3	35	2.04	0.68
	20	X	29	33	814	15	15	4	N	•	N	9	30	1.73	0.89
	20	χ	29	34	814	20	16	5	N	4	N	9	36	2.03	0.95
	20	X	29	35	814	16	19	5	N	•	N	8	35	1.96	0.95
	20	X	29	36	814	4	36	2	N	Ċ	N	10	40	3.70	1.42
	20	X	29	37	814	9	26	4	Ÿ	6	γ	13	35	0.70	
	20	X	29	38	814	1	34	1	N		N	9	3 <b>5</b>	2.42	0.66
	20	X	29	39	814	13	22	4	N	·	N	9	35	1.61	1.11
	20	X	29	40	814	••		•	.,	•	••	•			••••
	20	X	29	41	000	16	17	5	N		N	В	33	2.36	1.00
	20	χ	29	42	000	24	6	5	N		N	5	30	2.08	0.85
	20	X	29	43	000	2	38	1	N		N	5	40	2.22	0.71
	20	X	29	44	000	2	31	1	N		N	6	33	2.35	1.26
	29	X	20	1	814	11	27	4	N		N	7	38	1.13	0.63
	29	X	20	2	814	16	17	4	Υ	1	N	8	33	2.73	1.23
	29	X	20	3	814	14	21	4	N		N	7	35	1.77	0.86
	29	X	20	4	814	38	0	10	N		N	9	38	2.19	0.55
	29	X	20	5	814	13	19	4	N		N	9	32	2.31	0.82
	29	X	20	6	814	24	6	5	N		N	8	30	1.49	0.70
	29	X	20	7	814					•					
	29	X	20	8	814	8	23	2	Υ		N	2	35	2.40	1.30
	29	X	20	9	814	20	10	5	N		N	9	30	2.81	0.95
	29	X	20	10	814	13	27	4	N	•	N	7	40	2.03	0.65
	29	X	20	11	814	20	15	5	N	٠	N	7	35	2.54	0.79
	29	X	20	12	814	11	19	5	N	•	N	9	30	2.51	1.04
	29	X	20	13	814	2	33	1	Y	4	N	12	35	2.46	1.13
	29	X	20	14	B14	22	13	4	N	•	N	9	35	1.48	0.76
	29	X	20	15	814	35	0	10	N	•	N	8	35	2.08	0.87
	29	X	20	16	814	2	38	1	Y	2	γ	11	40	1.96	1.28
	29		20	17	814	29	1	9	N	•	N	10	30	2.32	1.12
	29	X	20	18	814	20	20	5	N	•	N	10	40	2.43	1.38
	29	X	20	19	814	33	2	9	N	•	N	7	35	1.73	0.89
	29	X	20	20	814	9	23	4	N	•	N	9	32	2.36	1.38
	29	X	20	21 -		12	23	4	N	•	N	11	35	1.58	1.12
	29	X	20	22	<b>B14</b>	12	23	3	N		N	9	35	1.72	0.89

					C 0	LUI	M N	N	0.	•		
CROSS	2	3	4	5	6	7	8	9	10	11	12	13
29 X	20 23	814	8	27	4	N	•	N	10	35	2.28	1.33
29 X	20 24	814	26	4	8	N		N	8	30	1.69	1.02
29 X	20 25	814	29	6	8	N		N	9	35	2.11	0.90
29 X	20 26	814	30	0	10	N	•	N	5	30	0.B1	0.45
	20 27	814	20	20	5	N	•	N	5	40	1.92	0.52
	20 28	814	16	29	5	Υ	3	Y	6	45	2.69	1.02
	20 29	814	16	24	4	γ	3	Y	9	40	2.93	1.38
	20 30	814	22	8	6	N	•	N	9	30	1.96	0.75
	20 31	814	18	22	5	Y	2	Υ	9	40	3.14	
	20 32	814	32	3	10	N	•	N	9	35	1.98	1.18
	20 33	814	14	26	4	N	•	N	9	40	2.71	1.25
	20 34	814	33	7	9	N	•	N	8	40	2.51	1.01
	20 35	814	40	0	10	N	:	N	9	40	2.99	1.04
	20 36	814	1	39	0	Y	1	N	10	40	2.40	0.91
	20 37 20 38	814 814	30 16	0 19	9 4	N N	•	N N	9 9	30 35	1.80	0.71
	20 39	814	30	0	9	N	•	N	9	30	2.13	0.92
	20 40	814	35	Ö	10	N	•	N	7	35	1.56	0.67
	20 41	000	19	21	5	N	:	N	9	40	1.16	0.81
	20 42	000	7	28	2	N	•	N	8	42	2.25	0.83
	20 43	000	3	37	1	N	Ċ	N	9	40	2.07	1.05
	20 44	000	2	28	1	Ÿ	2	γ	7	30	1.53	0.76
74 X 13		814	14	31	4	N		N	11	45	2.20	1.09
	35 2	814	14	21	4	N		N	10	35	1.59	0.49
74 X 13		814	18	22	5	N		N	10	40	1.39	0.58
74 X 13	•.	B14	7	33	3	γ	4	Υ	12	40	1.89	0.82
74 X 13		814	19	11	6	γ	1	N	9	30	0.95	0.49
74 X 13	35 6	814	3	37	1	γ	1	N	10	40	2.43	1.32
74 X 13	55 7	814	1	34	0	Υ	1	N	9	35	1.78	0.80
74 X 13		814	21	14	5	N		N	10	35	1.79	0.88
74 X 13		814	14	21	4	N		N	9	35	1.42	0.61
74 X 13		814	11	24	3	Υ	1	N	10	3 <b>5</b>	2.60	0.95
74 X 13		814	3	29	2	Υ	1	N	9	32	1.77	0.66
74 X 13	-	814	9	31	3	Y	2	N	10	40	2.30	0.77
74 X 13		814	4	36	2	γ	2	N	11	40	2.75	1.02
74 X 13		814	14	21	4	N	•	N	10	35	2.14	0.90
74 X 13		814	17 3	23 37	4	N Y	i	N	11	40	1.99	1.01
74 X 13		814 814	2	38	2 1	N		N N	10 11	40 40	2.46	0.87
74 X 13	,	814	9	31	3	N	•	N	9	40	1.93	0.86
74 X 13		814	11	29	3	N	•	N	9	40	2.56 2.11	1.11 0.75
74 X 13		814	12	18	5	Y	1	N	11	30	1.83	0.70
74 X 13		814	2	33	1	N		N	9	35	2.94	0.70
74 X 13		814	12	23	4	Y	2	N	12	35	2.78	1.28
74 X 13		814	9	26	3	Ϋ́	1	N	11	35	3.03	1.40
74 X 13		814	5	35	3	N		N	10	40	2.29	1.18
74 X 13		814	14	21	3	Υ	i	N	12	35	1.97	1.01
74 X 13		B14	17	21	4	N		N	9	38	2.04	0.99
74 X 13		B14	17	23	3	Y	2	Ϋ	9	40	2.47	0.82
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#	2	3	4	5	6	7	B	9	10	11	12	13
74 X 135	28	814	17	23	3	Υ	1	N	9	40	1.84	0.47
74 X 135	29	814	9	31	3	Υ	2	Υ	10	40	2.37	1.17
74 X 135	30	814	21	14	5	Υ	2	N	5	35	1.80	0.82
74 X 135	31	814	21	19	4	N		N	10	40	2.23	0.86
74 X 135	32	814	13	17	4	N		N	10	30	1.82	0.82
74 X 135	33	814	11	24	3	Υ	2	N	11	35	2.50	1.14
74 X 135	34	814	7	33	3	Υ	1	N	10	40	2.58	0.99
74 X 135	35	814	4	36	3	Υ	1	N	12	40	2.37	1.07
74 X 135	36	<b>B14</b>	7	30	3	Y	3	N	10	37	3.23	1.35
74 X 135	37	814	5	30	3	γ	1	N	9	35	2.43	0.76
74 X 135	38	B14	1	34	1	N	. •	N	12	38	2.60	1.24
74 X 135	39	814	21	9	7	N		N	13	30	3.65	1.59
74 X 135	40	814	6	29	3	Υ	1	N	9	35	3.69	1.15
, 74 X 135	41	000	1	39	1	Υ	1	N	11	40	3.15	1.03
74 X 135	42	000	3	32	2	N		N	9	35	2.38	0.78
74 X 135	43	000	3	33	2	N		N	10	35	2.61	0.69
74 X 135	44	000	2	33	1	Υ	3	N	11	35	3.96	1.28
135 X 74	1	B14	11	29	3	N	•	N	10	40	2.45	0.85
135 X 74	2	814	16	19	9	N		N	9	35	2.16	0.70
135 X 74	3	814	7	33	3	Υ	1	N	13	40	3.38	1.50
135 X 74	4	814	17	15	3	N	•	N	11	32	2.50	0.97
135 X 74	5	814	7	28	3	Υ	2	N	13	35	3.07	1.29
135 X 74	6	814	4	26	3	N	•	N	8	30	2.38	0.67
135 X 74	7	814	4	26	3	N	•	N	10	30	2.21	0.66
135 X 74	8	814	7	28	3	N	•	N	10	35	2.25	0.73
135 X 74	9	814	11	26	4	N	•	N	11	37	2.39	0.64
135 X 74	10	B14	4	27	3	N	•	N	9	31	1.96	0.53
135 X 74	11	814	11	19	3	Υ	2	N	12	30	2.39	1.02
135 X 74	12	814	12	20	3	N	•	N	11	32	2.70	1.24
135 X 74	13	814	13	21	3	Y	3	N	10	35	2.49	1.02
135 X 74	14	814	16	14	5	Y	3	Y	11	30	2.38	0.97
135 X 74	15	814	15	15	5	N Y	· 1	N	9	30	2.32	0.87
135 X 74 135 X 74		814 814	· 6	24 33	3 3	Y	, <u>i</u>	N N	10 12	30 42	2.16 2.79	0.80
135 X 74		814	4	33 26	3	N		N	9	30	2.17	1.10
135 X 74	_	814	5	25	3	N	:	N	12	30	3.25	1.33
135 X 74		814	16	17	3	Y	2	Υ	11	33	2.38	1.08
135 X 74		814	8	24	3	Ÿ	1	N	11	32	2.75	1.23
135 X 74		814	3	27	3	Ÿ	1	N	9	30	2.18	1.04
135 X 74		814	29	1	8	Ÿ	5	Υ	12	30	2.92	0.93
135 X 74		814	8	27	3	Ÿ	2	Ÿ	12	35	2.95	1.23
135 X 74		814	8	22	4	N	•	N	11	30	1.39	0.76
135 X 74		814	3	27	2	N	:	N	7	30	3.04	0.94
135 X 74		814	7	23	3	N	•	N	11	30	2.68	1.70
135 X 74		814	6	36	3	Υ	i	N	12	42	2.85	1.42
135 X 74		814	5	30	2	Ϋ́	2	N	14	35	2.64	1.56
135 X 74		814	15	19	3	N	-	N	11	34	2.71	1.08
135 X 74		814	11	21	3	γ	1	N	12	32	2.90	1.38
135 X 74		814	7	21	4	N	•	N	11	28	2.65	1.00

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	<b>#</b> 		2	3	4	5	6	7	8	9	10	11	12	13
135	Y	74	33	814	12	23	3	N		N	10	35	1.78	0.70
	X	74	34	B14			-		•	•				
	X	74	35	814	7	28	3	Υ	2	N	11	35	3.05	1.57
	X	74	36	814	11	19	4	Υ	1	N	11	30	2.84	1.48
	X	74	37	814	13	23	4	N		N	9	36	2.63	0.95
	X	74	38	814	6	24	3	Υ	1	N	10	30	1.91	0.76
	X	74	39	814	14	22	4	Υ	2	N	11	36	2.33	0.98
135	X	74	40	814	10	20	3	γ	1	N	11	30	2.15	0.85
135	X	74	41	000	24	6	8	N		N	11	30	2.41	1.09
135	X	74	42	000	16	14	5	N		N	10	30	1.85	0.85
135	X	74	43	000	15	17	4	N		N	10	32	2.48	1.36
135	X	74	44	000	2	33	2	γ	2	Υ	10	35	2.84	1.32
135	X	74	45	000	3	32	2	Υ	2	N	9	35	2.B1	1.03
, 135	X	74	46	000	4	31	2	N		N	6	35	2.25	0.75
37	X	185	1	814	20	20	4	N		N	9	40	2.47	1.47
37 )	X	185	2	814	24	6	5	N		N	9	30	1.78	0.93
37	X	185	3	814	35	5	10	N		N	9	40	2.12	0.97
		185	4	814	32	8	9	N		N	9	40	1.97	1.13
37 )		185	5	814	26	9	7	N		N	9	35	2.08	0.88
37 )		185	6	814	27	8	10	N		N	11	35	2.13	1.43
37 )		185	7	814	14	21	4	N	•	N	11	35	3.01	1.73
37 )		185	8	814	23	17	5	N	•	N	11	40	2.43	1.59
37 )		185	9	814	1	34	0	N	•	N	9	35	2.62	1.35
		185	10	814	15	20	4	Υ	3	N	11	35	3.05	1.77
		185	11	814	34	1	10	N	•	N	8	35	2.27	1.26
37 X		185	12 -		25	10	6	Υ	1	N	10	35	2.43	1.21
37 X		185	13	814	1	44	0	Υ	2	N	11	45	2.36	1.51
37 X		185	14	814	18	22	4	N	•	N	6	40	1.48	0.67
37 X		185	15	814	37	3	10	N	•	N	9	40	1.12	0.87
37 X		185	16	814	32	8	10	N	•	N	9	40	2.17	1.28
37 X		185	17	814	30	5	10	N	•	N	9	35	2.69	1.04
37 X		185	1B	814	16	24	4	N	•	N	7	40	2.12	1.04
37 X		185	19	814	35	5	10	N	,	N	10	40	2.09	1.28
37 X 37 X		185 185	20 21	B14	25	8	9	N		N N	10	33	1.76	0.82
37 X 37 X		185	22	814 B14	30 12	10 23	8 3	N Y	1	N	11 9	40 35	2.01 2.74	1.41
37 X		185	23	814	25	5	7	N		N	11	30	2.71	1.68
37 X		185	24	814	30	5	9	N	:	N	11	35	2.91	1.31
37 X		185	25	814	10	25	4	Y	1	N	11	35	3.43	1.74
37 X		185	26	814	33	7	10	Ņ	•	N	10	40	2.19	1.09
37 X		185	27	814	27	3	9	N	•	N	9	30	1.84	0.84
37 X		185	28	814	32	8	9	N	•	N	9	40	1.83	1.16
37 X		185	29	814	31	9	9	N	:	N	10	40	1.60	1.09
37 X		185	30	814	32	8	8	N	•	N	9	40	2.20	1.23
37 X		185	31	814	29	6	8	N	:	N	9	35	1.63	0.81
37 X		185	32	814	-,	5	J	.,	•	"	,	J		0.01
37 X		185	33	814	30	5	10	N		N	9	35	1.12	0.79
37 X		185	34	814	40	ō	11	N		N	9	40	2.02	1.11
37 X		185	35	814	34 .	1	10	N		N	9	35	1.72	0.72

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	oss	3										. <del></del> -		
	# 		2	3	4	5		7	8	<del>-</del> -	10	11	12	13
37	X 1	185	36	814	30	10	8	N		N	7	40	2.08	1.0B
		185	37	814	27	3	9	N	•	N	9	30	2.34	0.97
		185	38	814		77		v		M		75	7 04	
		185 185	39 40	814 814	2 12	33 23	1	Y N	1	N N	9 8	35 35	3.21 2.29	1.47
		85	41	000	14	21	4	N	:	N	6	35	1.27	0.76
		B5	42	000	34	6	8	Υ	2	Υ	8	40	1.32	0.95
		85	43	000	6	34	1	N	•	N	11	40	3.12	1.69
		85	44	000	11	29	2	Y	1	N	9	40	2.16	1.29
	X 1 X	85 37	45 1	000 814	1 19	44 16	0 5	Y Y	1 2	N N	9 9	45 35	1.79 2.01	1.23
	X	37	2	814	29	6	9	N		N	11	35	1.73	1.09
185	X	<b>37</b>	3	814	26	6	8	N		N	9	32	1.10	0.82
, 185		37	4	B14	14	19	4	N		N	9	33	1.62	0.95
185		37	5	814	8	22	3	Y	1	N	6	30	1.63	0.67
	X X	37 37	6 7	814 814	21 16	14 19	6 4	N N	•	N N	10 10	35 35	1.97 1.83	1.17
	X	37	8	814	22	8	8	N	:	N	7	30	0.92	0.60
	X	37	9	814	20	10	4	Υ	1	N	10	30	2.30	1.25
	X	37	10	814	24	6	8	N	•	N	9	30	1.96	0.65
	X	37	11	814	26	4	9	N	•	N	11	30	3.16	1.43
185 ) 185 )		37 37	12 13	B14 B14	10 12	25 1B	4 4	N N	•	N N	11 9	35 30	1.86	1.06
185		37	14	814	15	20	4	N	:	N	10	35	2.79	1.48
185 )		37	15	814	14	16	4	N	•	N	9	30	2.40	1.35
185 )		37	16	814	13	22	4	N	•	N	8	35	2.04	0.92
185 )		37	17	814	30	10	9	N	•	N	9	40	1.83	0.99
185 X		37 37	18 19	814 814	12 16	18 14	4 4	N Y	1	N N	9 9	30 30	2.42	1.23
185 X		37	20	814	10	20	3	Ϋ́	1	N	10	30	1.75	1.08
185 X		37	21	814	20	10	6	N		N	9	30		1.15
185 X		37	22	814	16	14	6	Υ	2	N	11	30	2.30	1.49
185 X		37	23	814	16	19	4	N	.:	N	9	35	2.26	1.27
185 X		37 37	24 25	814 814	18 28	12 2	7 9	N N	•	N N	11 9	30 30	1.60 1.71	1.35 0.70
185 X		37	26	814	30	5	8	Y	i	N	12	35	3.35	1.62
185 X		37	27	814	3	27	1	Υ	2	N	10	30	3.29	1.51
185 X		37	28	814	16	14	3	N	•	N	9	30	1.74	0.95
185 X 185 X		37 37	29	814	25	10	7	N	•	N	10	35	2.23	1.31
185 X 185 X		37	30 31	814 814	15 16	20 24	4 4	N Y	2	N N	10 11	35 40	2.50 2.66	1.73
185 X		37	32	814	24	9	7	N		N	9	35	2.59	1.25
185 X		37	33	814	7	23	3	N		N	11	30	3.56	1.67
185 X		37	34	B14	24	6	8	N	•	N	9	30	2.69	1.62
185 X		37	35	814	23	7	7	N	:	N	9	30	2.20	1.09
185 X 185 X			36 37	814 814	24 35	16 5	6 10	Y M	1	Y N	9	40	2.81	1.41
185 X			38	814	35 12	23	4	N Y	2	N N	10 11	40 35	2.32 2.54	1.16 1.97
185 X			39	814	21	9	7	, N		N	11	30	2.05	1.00

### CROSSES -- ARLINGTON CLOVER

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CE	ROS	35												
0,	#		2	3	4	5	6	7	8	9	10	11	12	13
185	X	37	40	814	11	19	4	N	•	N	10	30	1.98	1.11
185	X	206	1	814	6	29	3	N		N	8	30		
185	X	206	2	814	7	28	3	N	•	N	9	35		
185	X	206	3	B14	6	24	3	Υ	1	N	10	30		
185	X	206	4	814	15	15	7	Y	2	N	11	30		
185	X	206	5	814	11	29	6	N	•	N	10	40		
185	X	206	6	814	7	30	3	N		N	11	37		
185	X	206	7	814	7	33	3	N	•	N	9	40		
185	X	206	8	814	26	8	10	N	•	N	8	34		
185	X	206	9	814	18	15	8	N	•	N	7	33		
185	X	206	10	814	12	33	5	N	•	N	8	45		
185	X	206	11	814	23	9	10	N	•	N	8	32		
185	X	206	12	B14	28	4	10	Υ	2	N	9	32		
185	X	206	13	. <b>B14</b>	12	23	6	N	•	N	9	35		
185	X	206	14	814	14	18	5	N	•	N	9	32		
185	X	206.	15	814	11	24	6	Υ	1	N	9	35		
185	X	206	16	814	15	16	6	Y	1	N	10	31		
185	X	206	17	B14	30	0	11	N	•	N	9	30		
		206	18	814	17	18	8	N	•	N	8	35		
185	X	206	19	814	9	31	4	Υ	1	N	10	40		
		206	20	814	32	0	11	N	•	N	9	32		
185	X	206	21	814	11	24	7	N	•	N	8	35		
185	X	206		814	9	26	4	Υ	9	Y	9	35		
185	X	206	23		1	34	0	Υ	1	N	9	35		
		185	1	B14	5	25	2	N	•	N	9	30		
206		185	2	814	11	22	6	N	•	N	8	33		
206	X	185	3	814	15	20	6	Υ	2	N	10	35		
206		185	4	814	8	27	4	Y	1	N	9	35		
206	X	185	5	814	33	0	11	N	•	N	9	33		
206	X	185	6	814	22	13	9	Υ	2	N	10	35		
		185	7	814	16	24	7	N	•	N	В	40		
		185	8	814	9	26	3	N	•	N	9	35		
		185	9	814	28	2	10	Υ	2	N	10	30		
		185				32	4	N	٠.		8	48		
206		185	11	814	21	12	8	N	•	N	8	33		
206		185	12	814	4	26	3	N	•	N	9	30		
206		185	13	814	23	7	9	N	•	N	9	30		
206		185	14	814	10	20	7	Y	1	N	10	30		
206		185	15	814	12	23	7	Υ	2	N	11	35		
206		185	16	B14	13	19	7	Y	2	N	11	32		
206		185	17	814	18	12	7	Y	2	N	10	30		
206		185	18	814	19	16	7	Y	1	N	9	3 <b>5</b>		
206		185	19	814	21	9	7	N		N	8	30		
206		185	20	814	2	33	1	Y	2	N	10	35		
206	X	185	21	000	1	29	0	N	•	N	9	30		

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#		3	4	5	6	7	8	9	10	11	12	13
269 X 270	1	814	5	25	3	N		 N	10	33		
269 X 270		B14	8	27	2	N		N	9	35		
269 X 270		814	14	21	5	N	•	N	10	33		
269 X 270	4	814	20	10	8	N	•	N	8	31		
269 X 270	5	814	2	33	1	Υ	1	N	9	35		
269 X 270		814	26	9	10	N	•	N	8	35		
269 X 270	7	814	7	23	4	γ	2	N	10	30		
269 X 270	В	814	1	29	0	N	•	N	9	30		
269 X 270		814	6	24	3	Υ	2	N	11	30		
269 X 270	10	814	9	26	4	N	•	N	10	35		
269 X 270		814	29	6	10	Υ	1	N	10	35		
269 X 270	12	814	6	29	4	N	•	N	11	35		
269 X 270	13	814	2	28	0	N		N	11	30		
269 X 270	14	814	4	26	2	Υ	1	N	11	30		
269 X 270	15	814	12	18	6	N		N	9	30		
269 X 270	16	814	25	5	9	N		N	8	30		
269 X 270	17	814	13	17	6	N		N	8	30		
269 X 270	18	814	3	27	2	N		N	12	30		
269 X 270	19	814	9	26	5	Υ	1	N	10	35		
269 X 270	20	814	3	37	1	N		N	11	38		
269 X 270		814	30	5	10	N		N	7	35		
269 X 270	22	814	14	21	7	γ	1	N	9	35		
269 X 270		814	2	28	1	N		N	9	30		
269 X 270		814	8	27	4	N		N	9	35		
269 X 270		814	21	9	8	Υ	2	N	10	30		
269 X 270		814	2	28	1	N		N	10	30		
269 X 270		814	17	12	8	N		N	8	29		
269 X 270		814	14	15	7	Υ	2	N	10	29		
269 X 270		814	4	31	2	γ	1	N	10	<b>35</b>		
269 X 270		814	18	12	8	N		N	9	30		
269 X 270		B14	5	30	3	N		N	11	<b>35</b>		
269 X 270		814	9	26	5	N		N	9	35		
269 X 270		814	8	22	4	γ	1	N	9	30		
269 X 270		814				N	٠.	N	10	35		
269 X 270	35	814	7	28	6	N		N	8	35		
269 X 270		814	6	29	3	Y	2	N	11	35		
269 X 270		814	6	29	3	γ	1	N	10	<b>35</b>		
269 X 270	38	814	7	25	3	N		N	10	32		
269 X 270	39	814	11	19	4	N		N	7	30		
269 X 270	40	814	17	12	6	Υ	1	N	9	29		
269 X 270	41	000	25	10	9	N		N	8	35		
269 X 270	42	000	1	34	0	Υ	2	N	11	35		
269 X 270	43	000	2	28	0	N		N	9	30		
269 X 270	44	814	3	27	1	γ	1	N	9	31		
270 X 269	1	814	30	0	11	N		N	8	30		
270 X 269	2	B14	15	19	7	N		N	9	35		
270 X 269	3	814	4	31	2	Υ	2	N	10	35		
270 X 269	4	814	8	32	4	Ÿ	2	N	10	40		
270 X 269	5	B14	2	33	Ó	N		N	10	35		
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CROSSES -- FLOREX CLOVER

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CROSS											4.0	17
#	2	3	4	5	6,	7	8	9	10	11	12	13
270 X 269	6	814	2	33	0	γ	3	N	11	35		
270 X 269		814		29	4	Y	1	N	9	35		
270 X 269		814	7	28	4	Υ	2	N	11	35		
	9	814	12	23	7	N			8	35		
270 X 267	10	814	19	16	8	Ϋ́	1	N	9	35		
				11	9	N	•	N	8	35		
270 X 269	11	814	24					N	7	35		
270 X 269	12	B14	26	8	9	N						
270 X 269	13	814	7	28	4	Υ	2	N	10	30		
270 X 269	14	814	2	28	1	Υ	3	N	12	30		
270 X 269	15	814	9	24	5	N	•	N	9	33		
270 X 269	16	<b>B14</b>	6	24	4	Υ	1	N	10	30		
270 X 269	17	814	13	19	7	N	•	N	8	32		
270 X 269	18		3	32	1	N		N	9	35		
, 270 X 269	19	814		21	7	N		N	7	35		
270 X 269	20		5	32	2	N		N	9	37		
			8	32	4	N		N	9	40		
270 X 269	21						•			35		
270 X 269	22		4	31	2	Υ	2	N	10			
270 X 269	23	814		29		N	•	N	8	40		
270 X 269	24	814	2	33	1	Υ	1	N	9	35		
270 X 269	25	814	7	31	4	N	•	N	9	38		
270 X 269	26	814	6	29	4	Υ	2	N	11	35		
270 X 269	27	814	2	33	1	N		N	9	35		
270 X 269	28	814	12	23	6	N		N	8	35		
270 X 269	29	814	16	15	8	Ϋ́	1	N	9	31		
			3	37	1	N		N	9	40		
270 X 269	30									30		
270 X 269	31	814	15	15	8	Υ	1	N	7			
270 X 269	32	814	22	13	7	N	•	N	8	35		
270 X 269	33	814	14	16	6	N	•	N	9	30		
270 X 269	34	814	11	29	5	Y	1	N	9	40		
270 X 269	35	814	14	18	6	N		N	8	32		
270 X 269	36	814	9	21	5	γ	1	N	10	30		
270 X 269	37	814		25	4	γ	2	Ν	10	32		
270 X 269	38	814		34	0	N		N	9	35		
270 X 269	39	814			0				12			
270 X 269	40	814	2	34	1	Ÿ	1	N	11	36		
270 X 267	41	000	1	29	ô	N.		N	9	30		
		000		33	0	N	•	N	11	35		
270 X 269	42		2				•					
275 X 277	1	814	15	20	4	N		N	10	35		
275 X 277	2	814	7	25	3	N	•	N	10	32		
275 X 277	.3	814	20	20	6	N	•	N	8	40		
275 X 277	4	814	8	32	3	N		N	9	40		
275 X 277	5	814	12	28	5	Y	1	N	9	38		
275 X 277	6	814	14	21	6	Y	1	N	10	35		
275 X 277	7	814	9	31	4	N		N	10	40		
275 X 277	8	814	2	28	1	N		N	9	30		
275 X 277	9	814	11	29	5	N		N	8	40		
							•			35		
275 X 277	10	B14	11	24	4	N	•	N	8			
275 X 277	11	814	26	11	8	N	•	N	6	37		
275 X 277	12	B14	33	2	9	N	•	N	7	35		

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CROSS #	2	3	4	5	6	7	8	9	10	11	12	13
			<b>-</b>									
275 X 277	13	814	18	12	7	Υ	1	N	8	30		
275 X 277	14	814	24	11	8	N	•	N	8	35		
275 X 277	15	814	8	22	3	N	•	N	9	30		
275 X 277	16	814	12	23	5	Y	3	N	11	35		
275 X 277	17	814	7	28	3	N	•	N	10	35		
275 X 277	18	814	6	29	2	N	•	N	9	35 32		
275 X 277	19	814	28	4	7	N	•	N	7	32		
275 X 277	20	814	17	15	6	N	•	N	8 11	35		
275 X 277	21	814	13	17	6 4	N	•	N N	10	30		
275 X 277	22	814 814	9 21	21 12	7	N N	•	N	9	33		
275 X 277	23 24	814	14	18	5	N	•	N	8	32		
275 X 277 275 X 277	25	814	15	15	4	N	•	N	9	30		
275 X 277	26	814	9	26	3	N	:	N	10	35		
275 X 277	27	814	15	20	5	N	·	N	В	35		
275 X 277	28	814	32	2	8	N		N	6	34		
275 X 277	29	814	14	18	5	Ÿ	1	N	7	32		
275 X 277	30	814	11	24	4	Ň	·	N	8	35		
275 X 277	31	814	6	29	2	N		N	9	35		
275 X 277	32	814	12	28	3	Υ	3	Ν	10	40		
275 X 277	33	814	8	28	3	N		N	9	36		
275 X 277	34	814	9	21	3	N		N	9	30		
275 X 277	35	814	6	26	3	N		N	9	32		
275 X 277	36	814	15	20	5	γ	2	N	10	35		
275 X 277	37	814	19	11	7	N		N	10	30		
275 X 277	38	814	11	24	5	N		N	11	35		
275 X 277	3 <b>9</b>	814	13	22	5	N		N	9	35		
275 X 277	40	814	17	23	4	N	•	N	7	40		
275 X 277	41	000	21	15	6	N	•	N	8	36		
275 X 277	42	000	1	32	1	N	•	N	8	33		
275 X 277	43		3		1	Υ	1	N	9	35		
275 X 277	44	000	1	29	1	N	•	N	8	30		
277 X 275	1	814	19	11	6	N	.•	N	7	30 35		
277 X 275	2	814	28	7	9	N	٠.	N	6 9	35 30		
277 X 275	3	B14	7	25	3 3	N Y	2	N N	11	36		
277 X 275	4	814 814	6 12	30 18	4	Y	3	N	8	30		
277 X 275 277 X 275	5 6	814	7	25	3	N		N	9	32		
277 X 275	7	814	6	24	2	N	:	N	9	30		
277 X 275	8	814	10	25	4	Ϋ	1	N	9	35		
277 X 275	9	814	21	9	7	N	•	N	6	30		
277 X 275	10	814	14	21	6	Y	1	N	10	35		
277 X 275	11	814	13	22	5	, N		N	11	35		
277 X 275	12	814	15	25	5	Υ	3	N	12	40		
277 X 275	13	814	12	23	4	Ņ		N	9	35		
277 X 275	14	814	33	2	9	N	:	N	5	35		
277 X 275	15	B14	8	26	2	N		N	8	34		
277 X 275	16	814	16	14	5	N		N	7	30		
277 X 275	17	814	9	24	3	N		N	9	33		

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CROSS #	2	3	4	5	6	7	8	9	10	11	12	13
277 X 275	18	814	21	14	7	N		N	8	35		
277 X 275	19	B14	14	16	5	Ÿ	1	N	10	30		
277 X 275	20	814	21	16	6	N		N	9	37		
277 X 275	21	814	4	32	1	N		N	8	36		
277 X 275	22	814	8	27	3	Υ	2	N	12	<b>35</b>		
277 X 275	23	814	7	28	3	N		N	10	35		
277 X 275	24	814	27	5	8	N		N	9	32		
277 X 275	25	B14	6	30	3	N		N	9	35		
277 X 275	26	814	12	23	4	Υ	2	N	8	32		
277 X 275	27	814	11	29	3	N	•	N	8	40		
277 X 275	28	B14	1	29	i	N	•	N	9	30		
277 X 275	29	814	15	15	5	N	•	N	6	30		
277 X 275	30	814	7	22	3	N	•	N	9	29		
, 277 X 275	31	814	9	26	4	N	•	N	11	35		
277 X 275	32	814	7	28	3	N	•	N	10	30		
277 X 275	33	B14	11	22	3	N	•	N	7	33		
277 X 275	34	814	10	20	2	γ	2	N	10	30 75		
277 X 275	35	B14	13	22	2	Y	3	N	11	35 34		
277 X 275	36	814	14	20	4	N	•	N N	8 7	35		
277 X 275	37	814	14	21 23	5 5	N Y	i	N	9	35		
277 X 275	38	814	12 11	23 24	4	N		N	9	35		
277 X 275 277 X 275	39 40	814 814	1	29	1	N	:	N	12	30		
277 X 275	41	000	3	27	1	N	Ċ	N	9	30		
277 X 275	42	000	2	33	1	N	:	N	8	35		
277 X 275	43	000	3	27	2	N	•	N	10	33		
279 X 294	1	814	11	19	3	N	•	N	10	30	2.78	0.62
279 X 294	2	814	26	11	7	N	•	N	8	35	2.03	0.85
279 X 294	3	B14	14	26	4	Υ		N	9	40	1.83	0.74
279 X 294	4	814	16	11	3	N		N	9	37	1.88	0.63
279 X 294	5	814	16	16	5	N		N	10	32	1.55	0.63
279 X 294	6	814	30	6	8	Υ	3	Υ	11	36	1.99	0.72
279 X 294	7	814	20	12	4	Υ	1	Υ	11	32	2.43	0.97
279 X 294	8	814	27	6	9	N	٠.	N	10	33	1.28	0.21
279 X 294	9	814	15	25	4	N	٠	N	11	40	1.92	0.75
279 X 294	10	814	5	30	3	N	•	N	7	35	2.41	0.70
279 X 294	11	814	20	20	5	N	•	N	7	40	1.29	0.54
279 X 294	12	814	22	13	7	N	•	N	7	35	2.11	0.66
279 X 294	13	814	12	25	5	N	•	N	9	37	1.74	0.59
279 X 294	14	814	26	14	В	N	•	N	8	40	1.66	0.48
270 X 294	15	814	16	18	6	N	•	N	11	36	3.04	1.27
279 X 294	16	814	1	39	0	N	•	N N	9	40 40	2.41 0.72	1.25
279 X 294	17	814	31	9	9	N N	•	N N	6 5	40	1.75	0.52
279 X 294	18	814	29	11 24	9 3	N M	•	N	9	35	2.35	0.83
279 X 294 279 X 294	19 20	814 814	11 21	14	ა 6	N N	:	N	10	35	2.39	0.91
279 X 294 279 X 294	21	814	14	21	5	N	:	N	5	35	1.20	0.37
277 X 274 279 X 294	22	814	7	28	3	N	:	N	10	35	3.43	1.09
279 X 294	23	B14	14	23	4	N	:	N	11	37	2.20	1.09
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C	RO	SS									·			
	#		2	. 3	4	5	6	7	8	9	10	11	12	13
279	X	294	24	814	19	21	6	Υ		Y	13	40	2.53	1.14
279	X	294	25	814	14	26	5	N		N	11	40	3.44	0.99
279	X	294	26	814	16	14	6	N		N	10	30	3.59	1.03
279	X	294	27	814	21	19	В	N		N	10	40	2.08	0.87
279	X	294	28	814	16	24	5	N		N	9	40	2.07	0.BB
279	X	294	29	B14	26	11	6	N	•	N	10	3 <b>5</b>	3.45	1.13
279	X	294	30	814	22	18	8	Υ		Y	12	40	3.94	1.67
279	X	294	31	814	14	26	4	N	•	N	10	40	1.88	0.84
279			32	814	31	11	4	N	•	N	9	42	2.55	0.74
279			33	814	22	8	7	N		N	10	30	2.55	0.99
279			34	814	16	14	5	N	•	N	6	30	1.95	0.57
279	X		35	814	20	15	7	N	•	N	9	35	1.73	0.64
279			36	814	20	15	5	N	•	N	8	35	1.50	0.45
279			37	B14	13	20	4	N	•	N	11	33	3.73	1.39
279			38	B14	1	39	1	N	•	N	10	40	2.79	1.53
279		294	39	814	11	29	3	N	•	N	10	40	2.67	0.93
279		294	40	814	18	17	7	N	•	N	9	35	3.73	0.96
294			1	814	21	12	6	N	•	N	В	33	2.13	0.57
294			2	814	7	33	3	Y	2	N	10	40	3.43	1.37
294			3	814	1 B	15	5	N	•	N	6	35	1.27	0.42
294			4	814	2	38	1	N	•	N	11	40	3.39	1.04
294			5	814	30	10	9	N	•	N	7	40	2.26	0.75
294			6	814	15	20	4	N	•	N	12	35	2.33	0.93
294		279	7	B14	31	4	8	N	•	N	7	35	2.39	0.57
294	X	279	8	814	11	24	4	N	•	N	10	35	2.49	0.97
294	X	279	9	814	26	14	7	N	•	N	9	40	1.83	0.76
294	X	279	10	814	12	23	5	N	•	N	13	35	2.98	1.26
294	X	279	11	814	16	16	6	N	•	N	11	32	2.63	1.24
294	X	279	12	814	14	21	6	N	•	N	10	35		
294	X	279	13	814	12	18	5	N	•	N	11	30	2.41	0.70
294	X		14	814	14	21	3	Y	2	Υ	9	35	1.63	0.60
294	X		15	814	7	28	4	N	•	N	9	35	1.99	0.71
294	X		16	814	11 22	19 13	6	N	. •	N	6	30	1.97	0.23
		279	17	814			7	N		N	9	35	1.21	0.61
294		279 279	18	B14	40	5	9	Y	5	Y	12	45	1.94	0.95
274			19 20	814 814	18 22	12 18	8	N Y	4	N Y	5 11	30	1.46	0.53
294			21	B14	6	29	5 3	N		N	10	40 35	2.72 2.97	0.92
		279	22	814	15	15	ა 5	N	•	N	5	30	1.02	0.85
294			23	814	17	18	5	N	•		10	35		0.21
294			24	814	21	19	4	Y	•	N Y	11	40	2.25	0.85
294		279	25	814	32	0	9						2.6B	0.91
	X	279	26	814	21	14	4	N Y	4	N Y	6 13	32 35	0.95 3.80	0.39 2.06
294	X	279	27	814	20	10	5	Ϋ́	3	N	11	30		
294	X	279	28	814	9	26	3	N		N	9	35	3.22 2.94	1.37 0.84
294	Ŷ	279	29	814	6	24	3 4	N	•	N	6	30	2.74	0.92
294	X	279	30	814	7	28	3	Y	5	Y	12	35	2.71	1.44
294	Ŷ	279	31	814	14	21	5	N		N	7	35	1.59	0.61
294	X	279	32	814	1 B	12	3	N	:	N	7	30	2.22	0.44
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### CROSSES -- FLOREX CLOVER

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294	X 279	33	814	21	19	5	N		N	10	40	3.71	1.09
294	X 279	34	814	18	17	5	N		N	10	35	2.17	1.51
294	X 279	35	814	21	24	6	N		N	11	45	3.40	1.53
294	X 279	36	814	14	21	5	N		N	10	35	2.93	1.12
294	X 279	37	814	19	11	4	N		N	10	30	2.90	0.91
294	X 279	38	814	13	22	5	N		N	11	35	3.10	1.25
294	279	39	814	21	9	5	N		N	10	30	2.67	0.89
294	279	40	814	3	31	2	Υ	2	Υ	13	32	4.04	1.02
294	( 279	41	000	29	6	8	N		N	9	35	3.74	0.97
294 )	279	42	000	5	35	2	Υ	1	N	11	40	3.57	1.00
294	( 279	43	000	2	33	2	N		N	9	35	2.93	0.90

VII.3 APPENDIX 3

Normality tests on the Fusarium infection length (IL) measured from the inoculation site for the parental populations of the two red clover cultivars, over all inoculation groups and within each group.

	OVER THE TWO RED CLOVER CULTIVARS	ARLINGTON	FLOREX			
Variables	D:normal Prob > D	D:normal Prob >	D D:normal Prob > D			
IL	0.13904 < 0.01	0.14884 < 0.01	0.13649 < 0.01			
SQRT IL	0.07559 < 0.01	0.08954 < 0.01	0.06151 0.04			
LN IL	0.08824 < 0.01	0.09865 < 0.01	0.09371 < 0.01			
IL 814	0.10748 < 0.01	0.15678 < 0.01	0.07376 > 0.15			
SQRT 814	0.05484 > 0.15	0.07795 > 0.15	0.06354 > 0.15			
LN 814	0.10442 < 0.01	0.08749 > 0.15	0.13902 < 0.01			
IL 927	0.15718 < 0.01	0.16718 < 0.01	0.17340 < 0.01			
SQRT 927	0.09637 < 0.01	0.13385 < 0.01	0.09526 0.14			
LN 927	0.10355 < 0.01	0.13940 < 0.01	0.07411 > 0.15			
IL 959	0.16394 < 0.01	0.15775 < 0.01	0.17512 < 0.01			
SQRT 959	0.09631 < 0.01	0.11512 0.02	0.10744 0.04			
LN 959	0.07781 0.04	0.08929 > 0.15	0.08201 > 0.15			

NOTES: IL: infection length, NOT TRANSFORMED SQRT: square root transformation of IL

LN : logarithmic transformation of IL

814, 927, 959: Normality tests on each group of inoculation