



Evaluation of crown rust (*Puccinia coronata* f.sp. *avenae*) as a biocontrol agent for wild oats on San Clemente Island  
by Laura Diane Carsten

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology  
Montana State University  
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**Abstract:**

Biological control of weeds with plant pathogens is a relatively new field with much potential as an alternative to chemical weed control methods. San Clemente Island, the southernmost of the California Channel Islands, has been subjected to large scale grazing disturbance and subsequent weed infestation, particularly by wild oat species (*Avena fatua* and *A. barbata*). The endemic pathogen crown rust (*Puccinia coronata* f.sp. *avenae*) was selected as a potential wild oat control agent for island use. Crown rust infections rarely result in host death but instead reduce reproductive output of the host. Therefore, three questions were addressed in this study: 1) is it possible to establish crown rust infections on the island prior to onset of natural infections 2) will the crown rust be effective in reducing fitness of wild oat populations, and 3) will reduced fitness translate into a competitive advantage for the native grass species on the island. Three replicate sets of plots were established on the island, each set containing a plot treated with crown rust, a control plot, and a plot treated with fungicide. Disease assessments were taken in these plots and outside of the inoculated plots to a distance of 100 meters for two years in order to establish onset date and severity of disease, and spread of disease from these plots. Wild oat reproductive output from these plots was measured and compared for differences among plot treatments. An experiment was also undertaken in the MSU greenhouses to examine the competitive interactions between wild oats and a native island grass, *Stipa* (*Nassella pulchra*). Biomass and reproductive output from this experiment were analyzed with t-tests and with non-linear regression analysis. Results from the island field trials indicated that artificial inoculations were established in inoculated plots both years, two weeks earlier than onset of natural infections in 1997 though not in 1998. This may have been due to unusual weather patterns in 1998 that caused earlier onset of natural infections and precluded higher success of artificial inoculations early in the season.

Analysis of reproductive output from these field plots showed that reproductive biomass and seed number were significantly lower ( $P < 0.05$ ) in inoculated plots as compared to control and fungicide treated plots. Similarly, results from the greenhouse study indicated that wild oat biomass and reproductive output was significantly lower in rust than in no rust treatments ( $P < 0.05$ ), and that the resistant *stipa*'s biomass was significantly higher in rusted treatments ( $P < 0.05$ ). Parameters from the regression analysis showed that the competition coefficient for non-infected wild oats was higher, indicating that rust application does shift the competitive balance somewhat in favor of the *stipa*, although *stipa* is still at a disadvantage. The data point to the conclusion that crown rust, over time, could be an effective agent in reducing populations of wild oats on San Clemente, especially if combined with heavy revegetation of the island with *stipa*. The limiting factor for this strategy may be application methodology.

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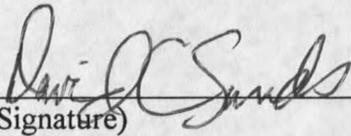
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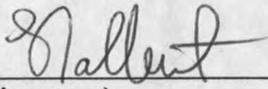
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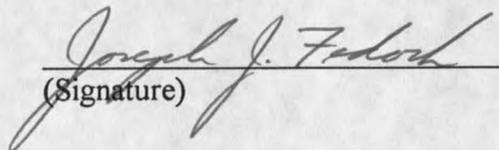
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## ABSTRACT

Biological control of weeds with plant pathogens is a relatively new field with much potential as an alternative to chemical weed control methods. San Clemente Island, the southernmost of the California Channel Islands, has been subjected to large scale grazing disturbance and subsequent weed infestation, particularly by wild oat species (*Avena fatua* and *A. barbata*). The endemic pathogen crown rust (*Puccinia coronata* f.sp. *avenae*) was selected as a potential wild oat control agent for island use. Crown rust infections rarely result in host death but instead reduce reproductive output of the host. Therefore, three questions were addressed in this study: 1) is it possible to establish crown rust infections on the island prior to onset of natural infections 2) will the crown rust be effective in reducing fitness of wild oat populations, and 3) will reduced fitness translate into a competitive advantage for the native grass species on the island. Three replicate sets of plots were established on the island, each set containing a plot treated with crown rust, a control plot, and a plot treated with fungicide. Disease assessments were taken in these plots and outside of the inoculated plots to a distance of 100 meters for two years in order to establish onset date and severity of disease, and spread of disease from these plots. Wild oat reproductive output from these plots was measured and compared for differences among plot treatments. An experiment was also undertaken in the MSU greenhouses to examine the competitive interactions between wild oats and a native island grass, Stipa (*Nassella pulchra*). Biomass and reproductive output from this experiment were analyzed with t-tests and with non-linear regression analysis. Results from the island field trials indicated that artificial inoculations were established in inoculated plots both years, two weeks earlier than onset of natural infections in 1997 though not in 1998. This may have been due to unusual weather patterns in 1998 that caused earlier onset of natural infections and precluded higher success of artificial inoculations early in the season. Analysis of reproductive output from these field plots showed that reproductive biomass and seed number were significantly lower ( $P < 0.05$ ) in inoculated plots as compared to control and fungicide treated plots. Similarly, results from the greenhouse study indicated that wild oat biomass and reproductive output was significantly lower in rust than in no rust treatments ( $P < 0.05$ ), and that the resistant stipa's biomass was significantly higher in rusted treatments ( $P < 0.05$ ). Parameters from the regression analysis showed that the competition coefficient for non-infected wild oats was higher, indicating that rust application does shift the competitive balance somewhat in favor of the stipa, although stipa is still at a disadvantage. The data point to the conclusion that crown rust, over time, could be an effective agent in reducing populations of wild oats on San Clemente, especially if combined with heavy revegetation of the island with stipa. The limiting factor for this strategy may be application methodology.

## LITERATURE REVIEW

### Introduction

San Clemente Island (SCI) has an interesting biological history. The island is inhabited by numerous endemic, rare or endangered species of plants (Junak et al. 1995), in addition to several endangered or threatened bird and animal species. However, the original state of the island has been highly altered due to human activities, including long term sheep and goat grazing (Kellogg & Kellogg 1994). These disturbances have led to massive colonization by exotic species, some of which have become numerous to the point of naturalization, such as *Avena barbata* and *A. fatua*, and others which are less numerous, but are still potential problem species, such as fennel and yellow star-thistle. The Navy now has full control of the island, and has extirpated the populations of grazing animals. This ownership also limits recreational use and other human impacts. The removal of the previous disturbance regimes offers an opportunity to restore the island to a closer resemblance of its pre-colonial state.

An essential aspect of this ecological restoration is weed control. The less numerous exotic dicots on the island have been controlled with some success by herbicide sprays and hand pulling, but grass species such as the wild oats are too abundant to be controlled in this fashion. Vegetation trend analysis has estimated the cover of the two wild oat species to be at approximately 60 % of all vegetation on the island. There are

some areas which remain relatively pristine, dominated by stipa (*Nassella pulchra*), a native bunch grass. Preservation of these areas, and increase of stipa, are priority goals for restoration, but there is little chance of this while the oats remain so numerous. The native grasses of the island flower and seed later than the wild oats, which gives them a reduced competitive advantage. Total elimination of wild oats is not necessary or probably even feasible, but reduction in fitness, including reduction in seed number and weight, and reduction in height and vigor of plants, would help the native grasses to regain a competitive advantage over the oats. Although competition and replacement on the island may be a process that takes many years, predictive models based on greenhouse trials can be developed to predict these factors (Sheley et al. 1996).

Weed control methods that have been proposed include fire, biological control using endemic species, and herbicide sprays. Fire is often cited as a useful tool for exotic weed control of grasslands, as many of these grasslands evolved under evolutionary pressure that included seasonal or periodic fires (Clark & Starkey 1990). Indeed, the island's periodic fire history has increased to a nearly annual cycle since the removal of feral goats from the island, which led to an increased load of dry annual grasses late in the growing season (Yatsko, personal communication). Despite this increase in late season fires, the oats still remain numerous, so it is unlikely that late season fires would contribute to wild oat control. Controlled burns early in the season may be a possibility for controlling wild oats, as this and other weedy species start their life cycle earlier in the season than many of the native grasses. However, wild oat tissue would still be quite green early in the season and may be difficult to burn. In addition, the unique island

biology forces other concerns to bear, including refuge sites for threatened wild life during burns, which may be limiting on an island, and the possibility of escaped controlled burns, which may impact sensitive plant species on the island. Clark & Starkey (1990) point out that many annual exotic species are not successfully controlled by burning. These species are prolific seed producers that are often well adapted to fire regimes, and while temporary reductions are sometimes seen, they quickly re-invade their previous habitats. Herbicide application has been used to control highly aggressive species with limited range on the island, but the oats are extremely abundant and widespread, and herbicide application across the island would be very costly as well as detrimental to sensitive species of concern on the island. Biological control seems to be the most feasible option for reduction of wild oats. The advantages of this type of control include targeting only the problem weed while leaving other plant species undisturbed, minimal impact on the fauna of the island, and low inputs once the species is established. If a mycoherbicide approach is developed in which pathogens are applied in a manner similar to that of chemical herbicides, inputs would still be lower than those for herbicide application. Using an endemic pathogen sidesteps the issue of introducing foreign species into this ecosystem, as well.

The rust fungus *Puccinia coronata* f.sp. *avenae* is endemic on the wild oat populations of the island, and is promising as a biological control agent of these species. Many successful biocontrol agents have been *Puccinia* sp. (Shishkoff and Bruckart 1996, Bruckart et al 1996, Parker et al, 1994). These rusts are often used in classical biological control, but could be successful as augmentative pathogens if ways can be identified to

increase the aggressiveness or start an early infection. The fungus causes a rather low percentage of mortality naturally, but if an epidemic can be started, increased mortality or at least reduction in fitness in the oats can be expected.

Beyond the practical considerations of weed control and ecological restoration, the island ecosystem offers an ideal setting for testing the interactions of a pathogen/ host system. Because the island ecosystem is essentially closed, movement of pathogens undergoing testing is restricted. Even though airborne pathogens such as crown rust are capable of traveling long distances, dispersion rates of such spores from an isolated setting would most likely be orders of magnitude lower in comparison to mainland dispersion rates. Also, genetic flow into island populations is largely restricted to genes already present on the island (Carlquist 1974), often leading to lower variability in these populations and thus potentially simpler interactions between host and pathogen. In addition, island systems, while still complex, are greatly simplified in comparison to their mainland counterparts. This makes it easier to identify and breakdown the interacting abiotic and biotic components that make up the biological system.

Islands are also subject to unique evolutionary pressures which may lead to phenotypes not seen on the mainland. Immigrating species are subject to the founder effect, which states that an island population founded by one or a few individuals is limited in it's initial genetic variation to the genotypes of these individuals (Carlquist 1974). However, once the immigrant has been established, rapid evolution almost always occurs. Mutations that may not be retained on the mainland often find a place in the island ecosystem, as different habitats, predation pressures, and competitors are present. This

leads to adaptive radiations of species as seen with Darwin's finches, or with the family Drosophilidae in Hawaii, which contains 400 species, a greater diversity of *Drosophila* than seen anywhere else in the world. These processes can occur rapidly. Five species of endemic banana-constant moths have evolved in Hawaii since the introduction of bananas to Hawaii only 1,000 years ago (Carlquist 1974). With the much faster generation time of pathogens, it is possible that strong evolutionary pressures have affected *Puccinia coronata* on San Clemente Island in the 200-400 years since wild oats became established. Therefore, island races may have special characteristics, including more or less virulence, altered or expanded host range, loss of alternate host followed by loss of recombination mechanisms, or other features conducive to island survival, although these effects may be lessened if infusions of mainland spores arrive with regularity.

### Description and History of Study Site

#### Description

San Clemente Island is the southernmost of the California Channel Islands, lying 79 kilometers off mainland California. The island is 58 km. long and flares in width from about 3.2 km. wide in the north to about 8 km. wide in the south (Junak et al. 1995). The northern end of the island is composed of a narrow, high plateau covered in grassland, which gradually rises in elevation southward to 599 m. at Mt. Thirst, approximately three quarters down the length of the island. The southern portion of the island is characterized by the high central escarpment with steep eastern canyons containing most of the island's

tree and shrub populations. The southwestern side of the island is composed of marine terraces covered in desert scrub, cholla and prickly pear cactus (Kellogg & Kellogg 1994). Most endemic plant species grow in the southern half of the island, particularly in canyon habitats, while the northern end of the island is more disturbed and contains higher numbers of exotic species.

### History

San Clemente Island was inhabited by Native Americans at the time of the first discovery of the island by the Spanish in 1542. The last Native Americans left the island in 1815. Goats were introduced to the island somewhere between these dates, and historical comments show that the goat population was well established by the 1840's. The U.S. annexed the island from Spain in 1848, and in 1868 the first permanent resident, a sheep rancher, arrived on the island. Goat and sheep populations were both numerous until 1934, when the Navy took over the island. Sheep were removed at this point, and the feral goat population exploded with no controls. A feral goat removal program was started in 1972, and goats were extirpated by 1992. Little is known of the botanical state of the island prior to Spanish colonization, but comparisons of botanical surveys in 1885 and the present show that the island had significantly higher tree and shrub coverage even in 1885 than now, and that exotic species have increased from an estimated 10 in 1885 to 99 in 1992 (Kellogg & Kellogg 1994).

Another exotic animal species of concern on SCI is the feral cat. House cats were introduced to the island at some point after European colonization, and cat populations

have subsequently exploded, with many individuals becoming feral. The critically endangered San Clemente Loggerhead Shrike is directly threatened by feral cat predation. Only twelve individuals existed in the wild as of 1997, with nest predation and habitat destruction listed as primary causes for population decline. The Channel Island fox, endemic to the Channel Islands and varying at the subspecific level between islands, is also a threatened species which may be negatively impacted by feral cats. Currently, a program is underway to extirpate feral cats from SCI.

#### Biocontrol of Weeds with Plant Pathogens

Suppression of exotic weeds on SCI may be achieved using biocontrol with plant pathogens. Biological control of weeds has been exercised with insects and other herbivores at least since the early 1900's, with several notable successes (Templeton 1982). However, the biological control of weeds with plant pathogens is relatively recent, emerging as a field only in the early 1970's (Freeman & Charudattan 1980). By the end of the first decade of such research, about 55 such projects were underway, with 42 of these investigating fungal pathogens and the rest involving bacteria, nematodes, and viruses (Templeton 1982). Thirteen of these were rusts in the genus *Puccinia*. As of 1993, more than 160 fungal pathogens had been studied as potential biocontrol agents (Yang & TeBeest 1993).

Classic thought on biocontrol strategies speaks of two major strategies: 1) classical biocontrol, and 2) the inundative or mycoherbicide approach, although these approaches

are not mutually exclusive. Classical control involves importation of an exotic agent that co-evolved with the weed of interest in the geographic area where the weed originated. With classical biocontrol, the aim is an initial release of the agent with the hopes that it will propagate and spread on its own, eventually establishing equilibrium with the weed at a population level below the current level of weed infestation (Watson 1991) with no further inputs. While this is desirable from a management perspective, these releases are usually irreversible, and unforeseen environmental consequences can result. Conversely, the inundative approach often involves agents that are present in the area of infestation in low levels. The potential control organism is isolated from a weed infested area, increased by rearing in a nursery or lab, then released back into the area of infestation in very large doses. Agents must be reapplied every year, which increases management costs but decreases environmental risk. The mycoherbicide approach is a modification particularly well suited for pathogens as opposed to insects, as the agent can be formulated and applied in the same fashion as chemicals (Charudattan 1991).

The two approaches described above are the most common in biocontrol, but a widening of the traditional paradigm opens up many other possibilities. 1) Agents with genetic modifications could be utilized in order to limit environmental persistence and/or increase virulence or lethality. 2) Pathogens that attack many weeds could be utilized, creating a more holistic approach to weed control. 3) Instead of inundative releases, focal point releases of inundative type agents could be used to start large scale infections. 4) Pathogens could be used as host-specific delivery systems, which selectively attack the weed of choice and deliver an herbicidal product. 5) Pathogens could be selected in the

lab for special characteristics, such as excess metabolite or toxin excretion, which may increase lethality. The possibilities of alternative biocontrol strategies are almost limitless when techniques such as genetic engineering are used in combination with the classic strategies.

For SCI, a blend of different approaches was undertaken. A pathogen already present on the island (crown rust) was chosen as an agent, and increased and released using the inundative approach. It was hoped that a classical effect would be seen as a result, with the pathogen increasing across the island from early infectious centers.

There are three main features considered when evaluating a fungal pathogen for release into a natural area. The first is the host specificity of the pathogen (Watson 1985). Whether a new organism is being introduced to an ecosystem or an existing organism is being increased, it is important that non-target plant species, particularly native and/or threatened species, are not negatively affected by the pathogen. Alternatively, it may be beneficial if a non-target species is infected by the pathogen to a very low degree, creating a reservoir of inoculum. Ensuring host specificity is often accomplished by selecting an obligate pathogen as a control agent, but can also be accomplished by genetic manipulation of a broad host range pathogen, either by creating an auxotrophic mutant or artificially narrowing the host range of the pathogen (Sands & Miller 1993). For SCI, an obligate pathogen was chosen where an extremely high level of host specificity exists.

The second feature considered is genetic variability, both of the pathogen and the target weed (Murray 1985, Barrett 1982). In a species or population with high genetic variability, new pathogen races may arise that are more virulent to the target species, a

desirable characteristic. However, this characteristic quickly becomes detrimental if the mutation involves a widening of host range (Leonard 1982). Genetic variability in an endemic pathogen with little ability to persist in the soil poses a much lower ecological risk. Conversely, a target weed with a narrow base of genetic variability may be easier to control, as genetic variation in the population may be a source for host resistance. Crown rust on SCI is suspected to lack the sexual cycle (Douglas, personal communication), limiting the variability of the pathogen, although spontaneous mutation and hyphal anastomosis are possible sources for variation. The genetic variability of the wild oat host has not been examined on SCI in detail, although we performed a resistance study that indicated uniform lack of resistance to crown rust in island populations. Clegg & Allard (1972) indicate that the California mainland populations of wild oats were introduced from the Mediterranean Basin during the colonization of North America, and are genotypically representative of the populations found in the Mediterranean. However, the populations on SCI may or may not show this representative diversity due to founder effects and the isolation of the island ecosystem (Nei et al. 1975).

The third feature of consideration when introducing a control agent is the lethality of the pathogen to the host. If the goal is reduction of the weed population, obviously the more lethal the pathogen the better. However, obligate pathogens often do not kill their hosts outright (Agrios 1988), so a trade off between host specificity and lethality is often necessary, although again, genetic manipulation of these pathogens is a possibility to increase lethality (Sands & Miller 1993).

### Implications of the Inundative Approach

The inundative approach has been characterized in the past as the manipulation of endemic fungal pathogens to create epidemic levels of disease (Charudattan 1988). Plants are attacked by a variety of pathogens, and evolutionary development of resistance and virulence in the host plant and the pathogen has led to many quasi stable associations in nature where low, endemic levels of disease exist in a given population or ecosystem (Walker 1969). Endemic levels of disease may stress individual plants but rarely cause enough damage to the host to be effective in controlling weed populations. Lack of initial inoculum is often a constraining factor for epidemic disease development (Shrum 1982), so application of inundative levels of the pathogen can often overcome this initial constraint.

The use of endemic pathogens, considered a tenet of the inundative approach, has many advantages over importation of foreign pathogens as control agents. An indigenous pathogen is already well adapted to the prevailing climatic conditions. This factor greatly improves the chances of successful epidemic disease onset and rapid disease progression (Charudattan 1988), as well as increasing the chances that a conducive infection window will present itself (Yang & TeBeest 1993). Secondly, an endemic pathogen is not subject to lengthy quarantine procedures to ensure safety. Because the pathogen is already in equilibrium in the target area, it is unlikely that the pathogen will attack non-target species in the region, if it does not already do so. Thirdly, there are risks associated with introducing any new organism into a system, as all possible ecological implications of an

introduction are not known until after the introduction occurs. The use of endemic pathogens already adapted to the system reduces risks of unforeseen and possibly detrimental ecological consequences.

Much of the past work with inundative control systems has been focused on creating sufficient levels of primary infections to cause epidemic disease levels. However, Yang & TeBeest (1993) indicate that secondary infection cycles are of great importance in establishing high levels of disease in the field. Often, an initial dose of inoculum may not be sufficient to infect large areas of the target weed, but secondary spore cycles and dispersal by the pathogen may be effective in creating epidemic disease levels later in the season. Field trials by Charudattan et al. (1985) showed that an initial disease rating of 5% soon after application of a pathogen could result in 90% mortality from secondary infections after about 5 weeks. Thus, the importance of pathogen build up and spread cannot be overlooked, and could be used as an alternate strategy when production of large amounts of initial inoculum is problematic.

#### *Puccinia* sp. as Biocontrol Agents: A Summary

The obligate nature and host specificity of the rusts has made them popular choices for numerous biocontrol projects (Tab. 1-1). Of the 21 projects published in the literature, 20 of these projects have been aimed at controlling dicotyledonous species. In addition, 15 of the 20 weeds targeted for control are members of the Asteraceae (Compositae) family (Two projects are aimed at *C. arvensis*, making for a total of 21 projects on 20

weeds). Efforts to control gramineous species with *Puccinia* sp. have been largely absent, with the exception of a pioneering project to control wild oats in Montana with *Puccinia coronata* in 1980 (Templeton 1982). Different forms of *Puccinia* are known to attack all species of grasses (Agrios 1988) and many dicots, so there is much unexplored potential in utilizing these species as biocontrol agents.

Table 1-1. List of published efforts to control weeds using *Puccinia* sp.

<u>Weed</u>	<u><i>Puccinia</i> sp.</u>	<u>Location</u>	<u>Reference</u>
<i>Acroptilon repens</i>	<i>P. acroptili</i>	Russia	Templeton 1982
<i>Ambrosia artemisiifolia</i>	<i>P. xanthii</i>	Russia	Templeton 1982
<i>Ambrosia trifida</i>	<i>P. xanthii</i>	N. America	Batra 1985
<i>Avena fatua</i>	<i>P. coronata</i>	Montana	Templeton 1982
<i>Carduus thoermeri</i>	<i>P. carduorum</i>	United States	Baudoin et al. 1993
<i>Centaurea calcitrapa</i>	<i>P. jaceae</i>	Maryland	Shishkoff & Bruckart 1996
<i>Centaurea maculosa</i>	<i>P. centaureae</i>	Canada	Clement & Watson 1985
<i>Centaurea solstitialis</i>	<i>P. jaceae</i>	Maryland	Shishkoff & Bruckart 1996
<i>Cirsium arvense</i>	<i>P. obtogens</i>	Montana	Templeton 1982
<i>Cirsium arvense</i>	<i>P. punctiformis</i>	U.S.	French et al. 1988
<i>Chondrilla juncea</i>	<i>P. chondrillina</i>	Australia	Templeton 1982
<i>Cyperus esculentus</i>	<i>P. canaliculata</i>	Maryland	Beste et al. 1992
<i>Cyperus rotundus</i>	<i>P. canaliculata</i>	Brazil	Barreto & Evans 1995
<i>Morrenia odorata</i>	<i>P. araujae</i>	Florida	Templeton 1982
<i>Oxalis</i> sp.	<i>P. oxalidis</i>	France	Templeton 1982
<i>Parthenium</i> <i>hysterophorus</i>	<i>P. abrupta</i>	Australia	Parker et al. 1994
<i>Senecio jacobaea</i>	<i>P. expansa</i>	California	Paul et al. 1993
<i>Senecio vulgaris</i>	<i>P. lagenophorae</i>	U.K.	Paul et al. 1993
<i>Xanthium canadense</i>	<i>P. canaliculata</i>	California	Templeton 1982
<i>Xanthium pungens</i>	<i>P. xanthii</i>	Australia	Templeton 1982
<i>Xanthium strumarium</i>	<i>P. xanthii</i>	North Carolina	Templeton 1982

The most widely publicized and successful of these projects was the effort against rush skeleton weed, *Chondrilla juncea*, with *Puccinia chondrillina*. *P. chondrillina*, an autoecious (single host) rust specific to rush skeleton weed, was first deployed in Australia

using the classical biocontrol strategy in 1971 (Leonard 1982). The fungus was first isolated in France, but these forms were avirulent on the Australian skeleton weed populations. A more virulent Italian isolate was found that attacks the most prevalent form of the Australian skeleton weed. This strain was released into the field after extensive host-range testing, and has spread successfully across the continent (Cullen et al. 1973). While *P. chondrillina* was extremely successful against the narrow-leaved form of rush skeleton weed, two other less common genotypes of the weed have begun to expand in range, replacing the narrow-leaved form (Hasan 1981). For complete control of this weed in Australia, new strains virulent on the other forms must be introduced, although Leonard (1982) indicates that the imported strain may become virulent on the two other forms of the weed through reverse mutation.

Efforts to use *Puccinia canaliculata* to control yellow and purple nutsedge (*Cyperus* sp.) are a good example of attempts to use *Puccinia* sp. with the mycoherbicide strategy. Use of this fungus has been explored to reduce weeds in tomato crops (Beste et al. 1992) and in subsistence agriculture in South America, but Barretto & Evans (1995) note that the techniques used to release and propagate this fungus may be incompatible with subsistence agriculture. However, the fungus may have potential as a classical agent for control of nutsedge pantropically.

*Puccinia carduorum* was released into western Virginia for control of musk thistle, *Carduus thoermeri* and related *Carduus* species, in 1987 (Baudoin & Bruckart 1996). 1992 surveys of rust spread indicated that the rust had spread across the eastern United States fairly extensively, more than 500 km. from the release site in some areas. However,

disease surveys during this time indicated low levels of disease on plants in most areas, with poor rust development in the fall and early spring, and lower infection on seedlings than on adult plants (Baudoin & Bruckart 1996). This illustrates that while dispersal rates of a pathogen can be high, this does not always translate into higher disease levels or better weed control in the field.

In addition to the above mentioned projects, there are five *Puccinia* species being investigated in the 1990's for potential classical release against weeds in the Compositae family: *P. punctiformis* against Canada thistle (*Cirsium arvense*) (Frantzen 1994), *P. jaceae* against star-thistles (*Centaurea* spp.) (Shishkoff & Bruckart 1993), *P. abrupta* var. *parthenicola* against Parthenium weed (*Parthenium hysterophorus*) (Parker et al. 1994), *P. expansa* against ragwort (*Senecio jacobaeae*), and *P. lagenophorae* against groundsel (*Senecio vulgaris*) (Paul et al 1993). None of these pathogens has been involved in a large scale, classical release to date, but all show high potential for success based on host-range studies, field trials, and/or epidemiological studies.

### Crown Rust

Crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is pathogenic on both domestic and wild oats. The pathogen has caused disease in oat crops for at least 200 years (Simons 1970), and has been studied extensively. Crown rust is an obligate, heteroecious, macrocyclic rust, with five spore stages: uredinial, telial, basidial, pycnial, and aecial (Dinoor 1988). The uredinial stage of the oats has been reported worldwide

where oats are cultivated, and occurs even more extensively on wild oats and volunteer grasses (Simons 1970). The fungus can reproduce asexually in repeating cycles of urediniospores on oat, but requires an alternate host for sexual reproduction. The sexual cycle begins with the production of overwintering teliospores, which produce basidiospores capable of infecting the alternate host. Pycniospores emerge on the upper leaf surface of infected alternate host plants, followed by emergence of aeciospores on the lower surface of the leaf, which in turn reinfect the gramineous host. Completion of the sexual cycle leads to higher genetic variation, new races, and stronger aggressiveness for the pathogen, making control more difficult (Fleischmann 1964). However, the sexual cycle occurs only in areas where an alternate host is present, which excludes areas of the world such as South America and Australia (Simons 1970). The list of known alternate hosts includes 30 species within the genus *Rhamnus* in addition to *Berchemia scandens*, *Elaeagnus commutata*, and *Lepargyrea canadensis canadensis*. *Rhamnus cathartica* (Buckthorn) has been the alternate host of the most importance in North America, particularly in Canada where this species is abundant (Chong 1996). In California and other regions of the southern United States, only uredia, telia, and basidia are present. An endemic species of rhamnus, *Rhamnus pirifolia*, occurs on SCI, but as of yet there is no evidence implicating this shrub in the life cycle of the pathogen.

Crown rust is known to be extremely host specific. The crown rust complex is divided into many sub-categories called *formae specialis* (f. sp.), or special forms, each of which is restricted to a single genus or species of host plant (Simons 1970), although some f. sp. can attack one or two species outside this range. Thus, *P. coronata* f. sp. *avenae* is

largely restricted to the genus *Avena*. This extreme host specificity makes this pathogen an ideal candidate for biological control of wild oats, particularly in natural areas where oats are not commercially grown, or where resistant cultivars are grown.

Virulence of *P. coronata* towards different varieties is controlled by one, two, or three independently inherited genes, and is a dominant trait in many cases (Dinoor et al. 1988). At least 29 such genes have been identified to date. Thus, genetically distinct races of crown rust can be identified based on their virulence. Because pathogenic interactions between the host and pathogen follow the gene for gene hypothesis, a differential set of near isogenic oat cultivars, each carrying one specific gene for rust resistance, was developed to aid in race identification (Dinoor et al. 1988). A single urediniospore from a rust pustule on an oat leaf is isolated and increased, then inoculated onto oat plants in this differential set. The reaction of each oat plant can range from highly susceptible to completely immune. Evaluation of the reactions on each oat cultivar creates an individual profile for the each crown rust race.

As indicated above, only the uredinial and telial stages are known in California; thus sexual recombination does not occur here. The races observed on SCI are highly diverse, which could indicate the presence of a sexual cycle, but other possible sources for variation include mutation, vegetative anastomosis, and parasexual recombination (Simons 1970). While teliospores are produced in California, the absence of the alternate host leaves no route for reinfection by the succeeding spore stages. Thus, the reinfection of the gramineous host is accomplished by uredinia. Winter temperatures do not pose a threat to uredinial survival in this part of the country; instead, oversummering becomes the limiting

factor. Uredinia survive hot summer temperatures on volunteer plants under leaking irrigation pipes and reinfect oat plants as they emerge in February or March. Crown rust is extremely temperature sensitive, with optimal successful infection of host tissue occurring in the narrow range of 18-20 C, although germination has been observed at temperatures as low as a few degrees above freezing, and as high as 30 C (Simons 1970). Temperatures on SCI generally fall within this outer range, and thus conditions are fairly amenable to rust survival and infection for a good portion of the year.

### Wild Oats

Both *Avena fatua* and *A. barbata* are serious problem weeds, both in agriculture and in natural areas. In North America and worldwide, wild oats are a serious problem in spring-seeded small grain crops (Whitson et al. 1996, Oates et al. 1983). Dormancy varies greatly between and among individuals from different localities across the United States (Toole & Coffman 1940), making predictions of emergence, and thus control, problematic. The length of viability in the soil has been estimated at anywhere from 3.7 to 6.7 years (Conn & Deck, 1995). In addition to causing loss in crops, wild oats are invasive species in grassland areas of coastal California and the Channel Islands, causing widespread habitat disturbance (Garcia et al. 1989). Thus, control of this species would be desirable in a variety of habitats.

Early work by Jain & Marshall (1967) indicated that many similarities exist between *A. fatua* and *A. barbata*. The species are morphologically similar, occupy similar

habitats, and were introduced concurrently to California during the Spanish period, between 200 to 400 years ago (Clegg & Allard 1972). Where the species distribution overlaps, pure and mixed stands of each species tend to form a mosaic across the landscape, with *A. fatua* occupying lower regions with heavier soils, and *A. barbata* tending to occupy more elevated grasslands with lighter soils (Jain & Marshall 1967). On SCI, *A. fatua* dominates the study sites, although *A. barbata* is also present. Both species are susceptible to crown rust. While both species are primarily self-pollinated, *A. barbata* is less genetically variable (Jain & Marshall 1967) and might possibly have fewer resistance genes against crown rust.

Efforts to control wild oat species have centered largely around the development of herbicides, cultural controls, and methods to break dormancy of the seed. Cultural practices that reduce wild oats in crops include use of weed free seed, summer fallow, and delayed seeding (Hunter 1983). While these strategies have been found to be effective in cropping systems, they are clearly not adaptable for natural areas. Breaking the dormancy of wild oat seeds is another potential control strategy. Methods suggested to induce germination include flooding fields prior to winter freeze (Hsiao & Quick 1983), and the use of soil stimulants, including ethylene, sodiumazide, and nitrates (Egley 1983). These methods could have value in a natural system to reduce wild oat seedbank, but control of emergent plants by biological or chemical means would still be necessary.

A wide array of herbicides has been developed to control wild oats. A partial list of these herbicides includes barban, benzoylprop, chlorphenpropmethyl, diclofop, difenzoquat, flamprop, and triallate. Each of these herbicides has a different mode of

action, time of application, and or chemical family, illustrating the wide array of possible options for wild oat control (Zimdahl 1993). However, all have some environmental persistence (Zimdahl 1993), and some may be injurious to non-target weeds, making wide-scale application to natural areas problematic.

Biocontrol efforts against wild oats have been minimal. Mortensen (1983) reported on a project at the Regina Research Station in which wild oat seed was analyzed for seed-borne fungi, but no correlation between pathogenicity and mortality was found. D.C. Sands worked briefly with *Puccinia* and *Pseudomonas coronata* in an effort to control wild oats, but growth was not reduced to any measurable extent (Mortensen 1983). Numerous species of fungi found to be pathogenic on oat seed were isolated from the spikelets of wild oats in Germany in 1964 (Mortensen 1983), many of which have biocontrol potential. We feel that the potential for biocontrol of this species has great potential and has been under explored in the past, and hope that the results of the SCI project will be useful in developing strategies control against wild oats in natural areas.

## COMPETITIVE INTERACTIONS BETWEEN WILD OATS (*AVENA FATUA*) AND STIPA (*NASSELLA PULCHRA*)

### Introduction

The native composition of the San Clemente Island grasslands is thought to have been dominated by the bunch grass *Nassella pulchra* (stipa, or purple needle grass) prior to European colonization. Consequent heavy sheep and goat grazing, in combination with repeated introductions of exotic grass species has resulted in domination of these grasslands by wild oats (*A. fatua* and *A. barbata*). Vegetation trend analysis has estimated the cover of these two species to be at approximately 60 % of all vegetation on the island (Kellogg & Kellogg 1994), although there are some areas which remain relatively pristine and dominated by stipa. Preservation of these areas, and increase of stipa, are priority goals for restoration, but there is little chance of this while the oats remain so numerous. Naturally occurring populations of *Puccinia coronata* f. sp. *avenae* (crown rust of oat) were identified on the island in 1994, and the pathogen is being investigated as a biocontrol agent for both species of wild oat. Although the obligate nature of crown rust confers the ability to reduce host fitness by sequestering resources, the pathogen seldom kills it's host outright. Therefore, interspecific competition between the weed and the desired species (stipa) becomes an important factor in predicting altered population levels of both species. Assumably, wild oats have been able to infest the island in part due to an ability to compete for shared resources or find a niche minimizing resource use overlap in the stipa dominated communities. The application of inundative

amounts of the rust pathogen to wild oat populations on the island may shift the ecological balance in favor of stipa. Therefore, comparison of interference between stipa and wild oats under conditions of epidemic rust compared to interference under conditions of no rust becomes an important question for predicting success of the biocontrol agent as a component of restoration.

To test this premise, a competition study between *N. pulchra* (stipa) and *A. fatua* (wild oat) was undertaken in greenhouse conditions. A variety of designs for this type of experiment have been explored in the past twenty years, including the additive design and the replacement series design. Both designs have been criticized because the effects of density vs. frequency of a species are difficult to separate, making analysis cumbersome (Radosevich 1990). In the early eighties, the addition series design was introduced (Connolly 1987). This experimental design estimates the fitness of both species at a variety of density combinations. This design also differentiates between the effects of intra vs. interspecific competition more clearly than other designs (Firbank & Watkinson 1985).

In this study, we used the equation developed by Firbank and Watkinson (1985). Analysis using this function allowed a direct estimate of the relative competitive ability and the potential to estimate niche differentiation.

### Materials and Methods

The experiment was planted in a greenhouse at Montana State University in late January, and harvested in April. Metal halide lights with 1000 watt bulbs extended the

days to simulate 14 daylight hours. Temperature was kept at 75 F daytime temp. and 70 F overnight temp. An addition series grid was established containing 25 pots with densities of each species at 0, 100, 500, 1000, or 2000 seeds per m<sup>2</sup>. A full factorial addition series including monocultures of both species was planted. The pot containing 0 plants was eliminated from the grid for a total of 24 pots. The grid was replicated eight times and randomized within the grid. Pots of size 20 x 20 x 32 cm. were used in order to restrict rooting as little as possible. Seeds were sown in a standard mix of greenhouse aquagrow soil and watered every four days for the first 4 weeks of the experiment, decreasing to once a week for the remaining 6 weeks. Of the eight replicates of the addition series, four were randomly chosen to be infected with crown rust. Infection consisted of even application of a 4:1 mix of talcum powder to crown rust urediniospores. Replicates targeted for infection were covered by a clear plastic tarp suspended 2 feet above the pot rim by wooden stakes. Ultrasonic humidifiers containing distilled water were used to simulate a 24 hour dew period under the tarps, after which the tarps were removed. Two subsequent re-infections of the replicates were undertaken at two week intervals by stirring up the inoculum present on the plants and exposing them to another 24 hour dew period. Final infection on target plants was visually estimated at 60% or greater coverage of the leaf area by the pathogen, which is similar to the late season levels observed in 1998 on San Clemente. Non target wild oat plants and sustained close to zero percent infection, and the resistant stipa sustained zero infection.

After 75 days, when the wild oats were at the soft dough to hard dough stage, the experiment was harvested. Live reproductive tiller number and mean plant height per pot

of wild oats were counted at harvest. Plant dry vegetative and reproductive weight were taken after a 48 hour drying period, and seed production of wild oat was counted (stipa produced no seed).

Data were fit to the Watkinson model (Firbank and Watkinson 1985) using non-linear regression (SAS 1992). Fitting data to the equations described by this model gives estimates for parameters that measure intra- and inter-specific competition between species (Cousens 1991). Regression analysis of competition experiments confers the ability to examine the shape of the response curve of the dependent to the independent variable, showing the effects of density on various treatments (Cousens 1991). Thus, the relative importance of competition between species in treatments with and without the pathogen can be separated by comparing the regression's fit to the data from each treatment. The Watkinson equations are as follows:

$$\text{In monoculture: } W = W_m (1 + aN)^b$$

where  $W$  = mean weight per plant,  $W_m$  = maximum weight of plant obtained in the experimental area,  $a$  = area required by the plant to achieve  $W_m$ ,  $N$  = density of the species, and  $b$  = the efficiency of resource utilization by the population.

$$\text{In two species mixture: } W_w = W_{mw} (1 + a(N_w + cN_s))^b$$

$$W_s = W_{ms} (1 + a(N_s + cN_w))^b$$

where  $W_w$  and  $W_s$  = mean weight per plant for wild oat and stipa respectively,  $W_{mw}$  and  $W_{ms}$  represent maximum weight obtained by wild oat and stipa respectively,  $N_s$  = density of stipa,  $N_w$  = density of wild oat, and  $c$  is the relative competitive ability, which describes mean equivalencies between species (Firbank & Watkinson 1985).

For parameter  $b$ , the expected outcome will be close to -1, indicating that the reciprocal yield law is in operation (Spitters 1983). Parameter  $c$ , the competition coefficient, is an estimate of impact and may vary between individuals as plants exhibit tremendous phenotypic plasticity in size even when grown under the same conditions (Watkinson 1986). Therefore, it can be useful to back up interpretation gleaned from these models with standard tests of differences between means, such as t-tests (Cousens 1991).

The Bartlett's Test and the  $F_{max}$  test (Sokal & Rohlf 1981) for homogeneity of variance were performed on data sets, followed by the extra sum of squares procedure to test for significant differences between data sets for parameters  $a$ ,  $b$ , and  $c$ .

Niche Differentiation (ND) was also determined for the two species system by calculating the ratio of the  $c$  parameters when fitting the data for each species (Spitters 1983). ND is a measure of how much overlap occurs between the ideal growing conditions for each species. A value for ND close to 1 indicates complete niche overlap, while values much greater or less than 1 indicate that the two species minimize draw on shared resources.

In addition to regression analysis, a t-test of mean plant parameters was performed between rust and no rust treatments of both species for the following parameters: wild oat height, wild oat reproductive weight per plant, wild oat seed weight, wild oat seed number per tiller, and above ground biomass (measured by weight) of both stipa and wild oat.

Concurrently, a growth analysis experiment was planted to ascertain maximum possible growth of each species when an individual is grown alone with no competition.

Ideally, growth of a single individual would be tracked and measured through the duration of the experiment, but as an individual must be harvested to measure biomass accumulation, this was not possible. Therefore, this experiment involves harvests at regular intervals, and replicate plants from each harvest are ranked according to size. Individuals from the same size class from every harvest were chosen to represent the growth of a single plant over time.

Four individuals of *A. fatua* and four of *N. pulchra* were planted in separate pots at the start of the competition experiment. These plants were subjected to the same watering regime, light conditions, and pot dimensions as those in the addition series. One plant of each species was harvested at two week intervals for the first six weeks, and a final harvest was made concurrently with the harvest of the addition series. Height, leaf area, and dry weight data were taken on each individual in every harvest. The experiment was replicated four times for a total of 32 seeds planted. Data were ranked according to size, and maximum plant size obtained was used as a measure for  $W_m$  in the Watkinson analysis. Some wild oat seeds failed to germinate, so some plant ranks do not reach the final harvest.

Results from the growth analysis were used to estimate the Relative Growth Rate (RGR) and the Net Assimilation Rate (NAR) of each species.

$RGR = \text{slope of } \ln \text{ total weight regressed over time in days.}$

$NAR = RGR/LAR$ , where  $LAR = LA/\text{biomass at the end of the growing period.}$

The RGR is a measure of growth relative to the original size, allowing comparisons of efficiency of biomass accumulation between species. While RGR is a useful measurement,

it assumes that all present biomass will contribute to the accumulation of further biomass; therefore NAR should be used to calculate the true carbon increase in the plant, as it is a measure of biomass accumulation relative to leaf area (Cousens 1991).

## Results

Comparison of means with a t-test showed significantly ( $p= 7.02 \times 10^{-11}$ ) lower reproductive output for wild oats under conditions of rust infection than no rust infection. This lower reproductive output included reductions in seed weight, seeds per tiller, and reproductive biomass. In addition, wild oat biomass was significantly ( $p= 0.003$ ) lowered by rust infection. Conversely, stipa biomass was significantly lower in the rusted treatments than the non-rusted treatments (Table 2-1).

### Monocultural Model

Analysis of the monocultural model showed a more or less negative hyperbolic response in both the stipa and the wild oat for biomass of individuals vs. genet density (Figs. 2-1 & 2-2). Conversely, a positive hyperbolic response was observed when plotting total weight per pot vs. genet density for both stipa and wild oats under conditions of both rust and no rust (Figs. 2-1 & 2-2). This effect is more pronounced for the wild oat data curves, although the presence of outliers distorts the shape somewhat in the rust treatment. The stipa curves contain a good deal of scatter, making these trends less visible.

Table 2-1. Mean values for various agronomic statistics on *A. fatua* and *N. pulchra*. Means were compared using a student's two tailed t-test. All means tested were significantly different between treatments at the P-value indicated.

Statistic measured	No rust mean values	Rust mean values	P-value
<i>N. pulchra</i> biomass	0.02 g	0.04 g	0.016
<i>A. fatua</i> biomass	1.40 g	0.88 g	0.003
<i>A. fatua</i> reproductive biomass	0.30 g	0.12 g	$7.02 \times 10^{-11}$
<i>A. fatua</i> mean biomass/seed	0.008 g	0.005 g	$7.08 \times 10^{-12}$
<i>A. fatua</i> seeds per repro. tiller	48.79	32.52	$5.23 \times 10^{-6}$
<i>A. fatua</i> height	85.0 cm	63.7 cm	$5.44 \times 10^{-5}$

Parameter estimates for  $a$  and  $b$  in wild oat were both significant for the rust treatment, but only parameter  $b$  was significant for the no-rust treatment. Neither parameter was significantly different between treatments. Parameter  $b$  was very close to -1 in both the rust and no-rust treatments (-1.5 and -1.2 respectively), indicating that the reciprocal yield law held.

### Two-Species Model

Fitting the two-species Watkinson model to the wild oat data yielded significant values (95% confidence interval did not include zero) for parameters  $a$ ,  $b$  and  $c$  for the no-rust treatment, and for parameters  $a$  and  $b$  for the rust treatment (Table 2-2). Locking in the significant values for  $c$  and  $b$  and refitting this equation still did not give a significant C.I. for parameter  $c$ .

The results of the Bartlett and F-max test for homogeneity of variances revealed

that the variances of mean wild oat weight between the two treatments were significantly different ( $\chi^2 = 16.45$  &  $F_{\max} = 2.29$ , respectively, with d.f. = 98), so the extra sum of squares procedure was not appropriate for testing for significant differences between parameter values for wild oats.

Table 2-2. Estimates of parameters for *A. fatua* resulting from fitting data to the Watkinson equation using non-linear regression.  $W_m$  for the rusted treatment = 6.39, and for no rust treatment  $W_m = 9.38$ .

Treatment	Parameter	Estimate	Lower C.I.	Upper C.I.	R <sup>2</sup>
Rust	a	3.647	2.388	4.904	0.372
Rust	b	-0.514	-0.706	-0.321	0.372
Rust	c	0.138	-0.135	0.411	
No Rust	a	0.750	0.199	1.301	0.630
No Rust	b	-0.756	-0.994	-0.518	0.630
No Rust	c	0.400	0.125	0.675	0.630

Parameter values for stipa resulting from the Watkinson fit were only significant for parameter *b* in both treatments (Table 2-3). Locking in values for parameters and refitting the equation achieved a significant (95%) C.I. for parameters *a* and *c* in the rusted treatment but only for *a* in the non-rusted treatment. The Bartlett and F-max tests showed that the variances of means were not significantly different for the stipa data ( $\chi^2 = 1.46$ ,  $F_{\max} = 1.27$ , respectively, with d.f. = 98) indicating that an extra sum of squares procedure was appropriate to use. The results of this procedure indicated no significant differences between parameters *a*, *b*, and/or *c* ( $F = 2.388$ , d.f. = [3, 149]) between the rust and no rust treatments.

Table 2-3. Estimates of parameters for *N. pulchra* resulting from fitting data to the Watkinson equation using non-linear regression.  $W_m$  for both treatments = 0.66

Treatment	Parameter	Estimate	Lower C.I.	Upper C.I.	R <sup>2</sup>
Rust	a	1.276	-0.178	2.731	
Rust	b	-1.063	-1.717	-0.409	0.430
Rust	c	0.388	-0.130	0.907	
No Rust	a	2.410	-0.922	5.743	
No Rust	b	-0.897	-1.522	-0.272	0.430
No Rust	c	2.289	-2.295	6.872	

The values for parameter  $c$  for wild oats were 0.138 for the rust treatment, and 0.400 for the no-rust treatment. The parameter  $c$  in this case represents the number of stipa plants that it takes to have an equivalent effect on wild oat biomass decrease as one wild oat plant. Therefore, a large  $c$  value indicates a lower competitive effect from stipa, so stipa had more of an effect on wild oats that were infected with rust. The values for parameter  $c$  for stipa were 0.388 under conditions of rust, and 2.289 under conditions of no rust, indicating that wild oats had more of an effect on stipa under conditions of rust. However, this result was not significant. Parameter  $b$  was consistently not significantly different from -1.0 for both the wild oat and stipa parameter estimates (Tables 2-2 & 2-3), indicating that the reciprocal yield law held true in this experiment, enabling calculations of ND. Parameter  $a$  was higher in the wild oat rusted treatment than in the non-rusted treatment (Table 2-2), indicating that the area required to achieve the maximum observed weight of a single wild oat plant grown in isolation is larger under conditions of rust.

Conversely, parameter estimates of  $a$  for stipa indicate that the area required for a single stipa plant to reach its maximum weight was larger under conditions of no rust.

Graphically displaying the predicted curves for individual biomass response showed the response predicted from the data (Figs. 2-3 & 2-4), that wild oat biomass under conditions of rust and no rust both fell with increasing stipa and wild oat density (Fig. 2-3). The shape of the curve shows a much steeper decline for wild oat weight under conditions of rust than under conditions of no rust, with wild oat weight rapidly decreasing at low densities of both stipa and wild oats (Fig. 2-3).

The stipa graphed in the same manner gave a similar response (Fig. 2-4), although the shape of both the rusted and non-rusted treatments was much steeper than the wild oat curves. Stipa biomass decreased with increasing wild oat and stipa densities, but this response was more marked in those treatments with no rust, indicating that rust treatment of oats leads to higher stipa biomass at all levels of intra and interspecific competition.

Niche differentiation was different in the rust treatments as compared to the no rust treatments. The ND value with rust was 0.355 for wild oats/stipa and 2.812 for stipa/wild oats, while ND without rust was 0.175 for wild oats/stipa and 5.723 for stipa/wild oats. The values in the rust treatment fall more closely about the number 1 than the no rust treatment values, indicating that niche differentiation shrinks when rust is applied.

### Growth Curve Analysis

Plots of  $\ln$  transformed biomass vs. time showed close to a linear relationship for both species, with the exception of one individual rank for the stipa (Fig. 2-5). The slope of these curves appeared similar, indicating a similar biomass accumulation rate.

Plots of  $\ln$  transformed leaf area vs. time showed somewhat different results. A near linear relationship exists for the wild oat, but the stipa showed a marked increase in leaf area accumulation rate after the second harvest (Fig. 2-6). At this point, the slope of the curve became much steeper for the stipa than the wild oat, indicating that leaf area accumulation occurs more quickly in stipa than in wild oat after an initial period of slow accumulation.

Table 2-4. Relative growth rate and net assimilation rates for *A. fatua* and *N. pulchra*. The symbol "a" indicates insufficient data to calculate statistic.

Plant Rank	<i>A. fatua</i>		<i>N. pulchra</i>	
	RGR	NAR	RGR	NAR
1	.1094	.0023	.1356	.0014
2	.1671	.0028	.1368	.0015
3	.1713	.0007	.1260	.0013
4	a	a	.1444	.0014
mean	.1448	.0020	.1448	.0014

Evaluation of RGR tended to support the result of similar biomass accumulation rate, as mean RGR was equivalent in both species (Table 2-4). NAR, which includes a measure of leaf area accumulation, was also similar between species, although slightly higher in wild oats. This result is in contrast to the steep slope of stipa leaf area















































































