INTEGRATED CONTROL OF CARDUUS THISTLES AND ECOLOGICAL STUDIES ON RHINOCYLLUS CONICUS FROELICH AND CEUTHORHYNCHIDIUS HORRIDUS (Panzer)

by

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I affectionately dedicate this dissertation to Mary Lou, my wife. Her support, self-sacrifice, and understanding have made this as much her accomplishment as mine.

We did it Babe!
ACKNOWLEDGEMENTS

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Section I
INTRODUCTION

Development of an integrated pest management (IPM) program for controlling Carduus thistles in pasturaneland requires information on the crop system, the biological control agents, and the control techniques intended for inclusion in the program. This basic information includes:

I. Crop system
   A. Cultural control
   B. Management practices

II. Chemical control procedures
   A. Type of herbicide
   B. Timing of application
   C. Application technique and rate

III. Host-biological control agent relationship
   A. Description and phenology of the hosts
      1. Carduus nutans L. (C. theormeri Weinmann)
      2. Carduus acanthoides L.
   B. Life history of the biological control agents
      1. Rhinocyllus conicus Froelich
      2. Ceuthorhynchidius horridus (Panzer)

IV. Compatibility of the control techniques
   A. Survival of herbicide-treated biocontrol agents
      1. Laboratory experiments
      2. Field trials
   B. Herbicidal impact on vitality of the biocontrol agents
   C. Herbicidal effect on biocontrol agent reproduction
      1. Sterility of males or females
      2. Egg viability

Much of this material is already available, including the life cycle of R. conicus, the application of impact of herbicides on thistles, the life cycle of C. horridus in Europe, and the use of management practices for reducing thistle infestations. However, some basic information necessary for the development of an IPM
program for *Carduus* thistles in pastures has not been reported. Therefore, experiments were designed to supply missing data on the phenology of the introduced insects in North America and the compatibility of the biological and chemical control techniques.

The literature review (section 2) contains much of the published information pertaining to the outline introduced in this section. The phenology of *C. horridus*, presented in section 3, was studied in order to determine which developmental stages of the biocontrol agent would be exposed to herbicide treatment. The next three sections document the compatibility of 2,4-Dichlorophenoxyacetic acid (2,4-D) with *C. horridus* and *R. conicus*. Finally, an IPM approach for *Carduus* thistle control is proposed.
Section II

LITERATURE REVIEW

The Crop System

Weeds in pastures compete with desirable plants for space, nutrients, moisture and light, resulting in a reduction of forage plant quality and yield (Klingman and McCarty 1958). Weedy thistles in the genus Carduus have been recognized as serious threats in pasture productivity since the 1950s, when intensive control efforts began in the United States (McCarty and Hatting 1975). Kates et al. (1972) reported that one thistle/1.49 m² reduced pasture yields an average of 23 percent. The Carduus species have the additional undesirable effect of limiting the use of infested areas by livestock or for recreational purposes because of the physical hazard presented by prominent spines on the leaves, stalks, and blooms (Higgins 1966).

The major techniques for controlling Carduus thistles include: cultural controls, management practices, herbicides, and most recently, biological control (Feldman et al 1968, Kok 1978, Puttler et al 1978). According to Klingman (1961) and Klingman and McCarty (1958), the lack of proper management practices (rotational or deferred grazing, water conservation, erosion control) and suitable cultural control techniques (mowing, fertilizing, reseeding) are often prime causes of pasture degeneration.

An analysis of management practices by Feldman et al. (1968) determined that continuously grazed pastureland was more susceptible to thistle development than rotationally grazed or non-grazed
pastureland. The total herbage (yield) increased with declining grazing levels; as the herbage increased, the number of thistle seedlings/acre decreased. The deleterious effects of over grazing pastures have been documented for musk thistle (Carduus nutans L.) by Higgins (1966) and for many other annual and perennial weeds by Klingman and McCarty (1958).

Cultural control methods, as well as management practices, offer an alternative to agricultural chemicals. McCarty and Hatting (1975) found that mowing musk thistle within two days of anthesis of the terminal blooms eliminated seed production, and regrowth was uncommon. However, mowing thistles four days after anthesis allowed significant amounts of seed to germinate. Also, the uneven maturity of thistle stands required more than one treatment per season. Kates (1962) suggested that low-level infestations could be reduced or eliminated by cutting thistles below the root crown with a shovel or hoe.

Pasture reclamation techniques such as no-till reseeding with competitive grasses and careful fertilization have been recommended by several authors (Klingman and McCarty 1958, and Kates et al. 1972). The efficacy of these methods was conditional upon implementation of good management practices (Klingman and McCarty 1958).

**Chemical Control Procedures**

Pasture and rangeland weed control was revolutionized by the development of inexpensive growth regulator herbicides capable of selective removal of broad-leaved weeds from grasses. The most commonly used chemical in this group is 2,4-Dichlorophenoxyacetic
acid (2,4-D) (Coartney and Kates 1968). This herbicide is rapidly translocated throughout the plant and interferes with crucial plant functions such as; respiration, synthesis and utilization of food, enzyme activity, stomate operation, turgor pressure maintenance, and most obviously, cell division (Klingman 1961, Coartney and Kates 1968, Rayle et al. 1969).

2,4-D has been recommended for use on Carduus thistles either singly, or in combination with similar phenoxy compounds including dicamba (2-methoxy-3,6-Dichloro-o-anisic acid), MCPA (2-methyl-4-Chlorophenoxyacetic acid), or silvex (2-2,4,5-Trichlorophenoxy propionic acid) (Kates 1962, McCarty 1964, Higgins 1966, McCarty and Hatting 1975, Kates et al. 1979). Most authors agreed that plant maturity affected herbicide efficacy; larger plants were less susceptible. However, timing of application was critical because best results were obtained by applying herbicides when thistles were small but actively growing. Spring and fall applications of 2,4-D at rates of 1.1 kg/ha to 2.2 a.i. kg/ha reduced Carduus species (Kates 1962, McCarty and Hatting 1975), but several years of herbicide treatment may be required to depress thistle infestations to a level suitable for cultural controls (Higgins 1966).

Description and Phenology of the Host Plants

Carduus, Cirsium, Silybum, and Onopordum are the primary genera in the family Asteraceae (= Compositae) which serve as hosts for C. horridus and R. conicus (Hoffmann 1954, Zwölfer 1965, and 1967, Dunn 1978). Although there are many representatives of these genera
in North America, only *Carduus pycnocephalus* L. (Goeden 1974), *C. nutans* (Kok 1975, Rees 1977), *C. acanthoides* L. (Kok et al. 1975), *Cirsium arvense* (L.) Scop. (Rees 1978), and *Silybum marianum* (L.) Gaertn. (Goeden and Ricker 1977) have been targeted for intensive biological control projects. In Virginia, only *C. nutans* (musk thistle) and *C. acanthoides* (plumeless thistle) have produced economic losses which justify a major biological control effort (Dunn 1976, Kok 1978).

Currently, both *C. nutans* and *C. acanthoides* are undergoing taxonomic revision. According to Boldt and Campobasso (1978), most of the thistles in the United States presently designated as *C. nutans* are actually *C. thoermeri* Weinmann. Confusion developed when *C. nutans* was split into subspecies (Mulligan and Frankton 1954) and then two subspecies, *C. nutans* ssp. *leiophyllus* (Petrovic) Arenes and *C. nutans* ssp. *macrocephalus* (Desfontaines) Fiori, were elevated to species status as *C. thoermeri* and *C. macrocephalus* Desfontains, respectively. Both of these species exhibit the constricted involucral bracts that Linnaeus used to define that original *C. nutans*, but vary in the shape of the bracts, pubescence of leaves, and color and form (McCarty and Scifres 1969, Boldt and Campobasso 1978). A small sample of specimens from Virginia have been identified as *C. thoermeri* (Boldt and Campobasso 1978).

Additional complications have arisen from the hybridization of *C. nutans* (resembles *C. thoermeri*) and *C. acanthoides*. Moore and Mulligan (1956) determined that chromosome numbers for *C. acanthoides* (2n = 22) and *C. nutans* (2n = 16) were clearly different. However, hybrids of these two species produced offspring containing chromosome
complements relating to all the intervening values (2n = 17-21). An index was developed by these authors to differentiate among the hybrids, but lack of use by other authors indicates that the index has not been generally accepted. Additional taxonomic confusion occurred when Mulligan and Frankton (1954) split C. acanthoides into three subspecies.

The life cycles for C. nutans and C. acanthoides are similar. Both species develop as biennials (Fernald 1950, Lacefield and Gray 1970), annuals (Carlson 1968, McCarty et al. 1969), or winter annuals (McCarty et al. 1967). McCarty and Scifres (1969) stated that variability in growth rates and germination are responsible for the diverse life histories, but these thistles generally act as winter annuals or biennials. Descriptions of the thistle life stages and occurrences have been reported for several geographic locations in North America (Fernald 1950, Lacefield and Gray 1970, McCarty et al. 1967, McCarty et al. 1969, McCarty and Scifres 1969).

Life History of the Biological Control Agents

The life cycle of R. conicus in both Europe and the United States has been documented (Zwölfer 1967, Surles 1972, Goeden 1974, Boldt and Campobasso 1978, Puttler et al. 1978, Rees 1978). Eggs are laid on the bracts and stems of thistle blooms, and larvae feed in the recepticle beneath the developing seeds. The larvae pupate in the blooms, and emerge as adults by mid-summer. These adults migrate to overwintering rosettes. C. nutans and C. acanthoides serve as hosts for this species in Virginia (Kok 1978).
Syncronization and impact of *R. conicus* on thistles have been documented in several states (Rees 1977, Goeden 1978, Surles and Kok 1978). Although *R. conicus* is well synchronized with *C. nutans*, impact of this weevil was reduced by the extended blooming period of *C. acanthoides* (Surles and Kok 1977, 1978). Attempts to increase the insects' ovipositional period through sequential releases of adults were generally unsuccessful (Surles and Kok 1976). This technique was effective in field cage tests, but fecundity was reduced by prolonged captivity and subsequent larval survival diminished due to insufficient developmental time.

Establishment of *R. conicus* on either thistle species was enhanced by releases made in the spring (prior to oviposition), rather than in the summer or fall (Kok 1974, Surles et al. 1974). In Virginia establishment of this weevil has led to the successful suppression of *C. nutans* (Kok and Surles 1975).

A thorough knowledge of the life history of *C. horridus* in Europe has been hindered by the indistinct taxonomic designation of this insect. *C. horridus*, originally described as *Curculio horridus* by Panzer (1801), commonly occurs under several synonyms in the literature. *Ceuthorhynchus spinosus* Goeze, described in 1771, was first listed as a synonym for *C. horridus* in 1871 (Gemminger and de Harold 1871). However, this specimen was later incorrectly synonymized with *Ceuthorhynchus troglodytes* F. (Everts 1903), and *C. horridus* became the accepted name.

Additional confusion about the taxonomic status of this weevil developed during the 1800s. In 1854, du Val placed *C. horridus* in
the genus *Ceuthorhynchidius*, but the species was subsequently included in the genus *Ceuthorhynchus* by Bedel in 1887. This apparent mistake was eventually discovered, but *Ceuthorhynchidius horridus* is still occasionally published as *Ceuthorhynchus horridus* (Harris and Zwölfer 1971). Unfortunately, when Schultz (1902, 1905) reinstated *C. horridus* to the genus *Ceuthorhynchidius*, the genus name was misspelled as *Ceuthorrhynchidius*, and was not an intentional revision of the genus. The double 'r' and single 'r' spellings of *Ceuthorhynchidius* have occurred ever since (Hoffmann 1954, Dieckmann 1972, Trumble 1977, Boldt 1978). Colonelli (1979) recently suggested that the genus *Ceuthorhynchidius* be changed to *Trichosiocalus* to help alleviate confusion.

In spite of the taxonomic chaos, the life history for *C. horridus* has been documented in Europe, but not in North America. The life cycles vary with location in Europe (Hustache 1923, Frick 1969), but follow the general pattern described below. Adults oviposit in the midribs of thistle leaves, and upon eclosion the larvae migrate to the crown of the rosette. Feeding is completed during the third instar, and larvae move into the soil to pupate. Adults feed immediately after emergence, and then move to rosettes suitable for oviposition. In Europe, this species has one generation annually. The life history of *C. horridus* in Virginia and comparisons with various life cycles of the species from Europe are presented in this dissertation.

Because of the taxonomic confusion surrounding *C. horridus* and the use of this species for biological control, a bibliography for
this insect has been categorized by major interest areas, and is presented in Table 1. The numbers shown in the table refer to references listed in the appendix.

Compatibility of the Control Techniques

Many studies are available on the impact of pesticides on arthropod biocontrol agents (Croft and Brown 1975), but few deal with the effects of herbicides on beneficial insects. The effect of 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) on a carabid predator of springtails is a notable exception (Eijackers 1978a). In this study, carabids treated with 2,4,5-T, placed on treated surfaces, or fed 2,4,5-T treated springtails showed reduced activity and longevity.

Eijackers (1978b, 1978c) also examined the impact of 2,4,5-T on plant (springtails) and detritus feeding arthropods (isopods). Exposure to 2,4,5-T resulted in reduced survival and an avoidance response to treated surfaces. However, in feeding tests, isopods preferred herbicide sprayed black cherry leaves to untreated leaves, but survival was not adversely affected. Eijackers speculated that the 2,4,5-T treatment may have caused structural changes in the food, including; a high protein content, a low C/N ratio, a looser leaf structure, and reduced penetration resistance.

There has been no work on the compatibility of herbicides with C. horridus, and very little on R. conicus. The few studies available are discussed in the applicable sections of this dissertation, where specific comparisons are drawn.
Table I. A Categorized Bibliography for *C. horridus.*

<table>
<thead>
<tr>
<th>Category</th>
<th>Biologically Oriented Papers</th>
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<td>Detailed biology or description</td>
<td>21,42,57,74,77,78,79,82,83</td>
<td>1,31,32,51,68</td>
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<tr>
<td>Partial biology or description</td>
<td>7,9,14,35,40,41,56,64,75,76,81</td>
<td>3,13,17,19,22,26,43,45,53,54,55,62</td>
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<td>With keys separating <em>C. horridus</em> from related spp.</td>
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<td>1,4,13,17,19,31,32,33,43,53,54,55,69,70</td>
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<td>Foreign exploration for <em>C. horridus</em></td>
<td>7,9,15,24,64,65,85</td>
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<td>Host specificity of <em>C. horridus</em></td>
<td>15,35,82,84</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Release and establishment for biocontrol</td>
<td>37,38,40,41,78,79</td>
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<td>Mention only</td>
<td>5,8,10,18,20,25,27,28,36,39,47,49,58,72,80,86</td>
<td>6,11,12,23,29,34,44,46,48,52,59,61,66</td>
</tr>
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*References not seen by author: 2,30,50,63,67,71,73; reference 17 does not mention *C. horridus*, but describes the genus. Numbers refer to references listed in Appendix A.*
Section III

CEUTHORHYNCHIDUS HORRIDUS (COLEOPTERA: CURCULIONIDAE):
LIFE CYCLE AND DEVELOPMENT ON CARDUUS THISTLES IN
VIRGINIA
Introduction

The life cycle of *C. horridus* in central Europe (Hustache 1923, Hoffmann 1954, Auber 1960, Scherf 1964) differs from that in southern Europe (Frick 1969). Although weevils in both locations produce one generation annually, their life histories are substantially different. In central Europe, oviposition occurs from the middle of May through June. Newly eclosed larvae migrate to the rosette crowns and feed until pupation in July and August. Adults emerge in September and subsequently overwinter. In southern Italy, oviposition occurs from the middle of December through early March. Larvae develop for approximately two months, pupate, and emerge as adults from April through June. These weevils apparently undergo an aestival diapause during the hot summer months and resume feeding in late fall or early winter.

Laboratory-reared progeny of *C. horridus* adults imported from southern Italy were released in Virginia in 1974 and 1975 (Kok and Trumble 1979). Establishment of the weevils was monitored closely between 1975-1978 and a study on their life cycle in *Carduus* thistles was conducted.

Materials and Methods

Five of the 1974/1975 release sites of *C. horridus* from which the weevil was recovered were monitored by examining crowns for feeding marks and larvae in the spring and fall of 1975-1977. Three sites (Montgomery Co. [Prices Fork], Pulaski Co. #1 [Route 611], Pulaski
Co. #2 [Belspring]), were selected for weekly observations in 1978, beginning with snow melt in March through the first week in August. Subsequently, the Pulaski Co. sites were monitored at least every two weeks through January 1, 1979. Details of releases and their locations were reported earlier (Kok and Trumble 1979).

Several sampling techniques were used to survey *C. horridus* populations during 1978. Occurrence of the egg stage was determined by weekly dissections of 50 leaves from 25 randomly selected musk thistle rosettes (*Carduus nutans* L.) and/or plumeless thistle rosettes (*C. acanthoides* L.). Larval counts were based on 15-minute surveys (approximately 20 plants) at each of the sites, and adults were studied by examining thistles (50 plants) and the ground immediately beneath them. Adult feeding activity was measured in the fall and winter by counting fresh puncture marks in 50 leaves from 25 randomly chosen rosettes.

**Results and Discussion**

The temporal distribution of eggs, larvae and adults of *C. horridus* in Virginia (Fig. 1) indicates a seasonal life cycle similar to that of weevils in southern Europe. Although the snow and the cold winter temperatures in southwestern Virginia resemble conditions of central Europe, the onset of oviposition was consistent with observations made by Frick (1969) from populations of *C. horridus* near Rome, Italy. The main deviation was that oviposition continued for an additional month (through early April) in Virginia. Thus, the
Fig. 1. Occurrence of eggs, larvae and adults of C. horridus on Carduus thistle in Virginia.
seasonal life cycle appears to be genetically controlled rather than environmentally influenced.

The first instars (n = 27) were active in late December and some (n = 12) were observed feeding on rosettes which were partially encased in ice. Larval activity continued through late May. Although snow cover prevented observations between early January and March 15, 1978, first instars were detected immediately after the snow melted. All three larval instars were found during the last two weeks in April, but peak abundance of the first two instars occurred in mid-April; third instar populations peaked during the first half of May (Fig. 2). However, early instars were observed at two other sites in early June of 1977 and 1978. This could have due to the slower larval development in higher elevations (Russell and Giles Co.) as compared to that at lower elevations (Montgomery, Pulaski or Warren Co.). Because the pupal stage is sensitive to disturbance (Kok et al. 1975) and develops underground, large-scale pupal sampling programs were not carried out.

Teneral adults (n = 611), emerging from mid-May through the middle of June, were usually found on upper leaves and blooms of flowering thistles. Peak abundance of adults occurred between early and mid-June, when adults were collected for biological studies. Collection was relatively easy at this time; yields at two sites were 454 weevils producing an average of 43 adults/man-hour of collecting. The average sex ratio was (♀:♂) 1:1.18. These collections represented an average population increase in excess of 200% over original adult releases after three years. After June 25, the adults were difficult to
Fig. 2. Relative abundance of C. horridus larvae based on weekly surveys at three established sites.
locate and from early July until late September no weevils or fresh adult feeding marks were found. This observation suggests an aestival diapause like that observed by Frick (1969) in _C. horridus_ populations in southern Italy. Termination of adult diapause during the last week of September was marked by increased feeding (Fig. 3). The rapid increase in high feeding levels observed at site 2 (Belspring) versus site 1 (Route 611) could be attributed to a more southern exposure and less shading. Feeding activity remained high until mid-December.

Some developmental stages of _C. horridus_ appear more suitable for population studies than others. Dissecting leaves for eggs was tedious and inefficient; because eggs were found in less than 1% of the samples' leaves, prohibitively large numbers of leaves would be required to accurately assess population density. First and third instars were easily detected; first instars produced characteristic damage symptoms (serpentine skeletonized trails marked with black frass) in young leaves and third instars hollowed out conspicuous cavities in the crowns of rosettes. However, second instars usually burrowed into the rosette crowns and were difficult to find. This explains why fewer second instars were found than the other two instars (Fig. 2).

Searching for adults among rosettes proved time-consuming and produced variable results. Fewer weevils were recovered during adverse weather conditions (rain, sleet). Newly emerged adults were easily collected on flowering thistles from mid-May through late June except on days with strong winds (in excess of 25 km/h) or rain.
Fig. 3. C. horridus feeding at two release sites in Pulaski Co., Virginia, during fall and winter of 1978.
Based on these observations, population studies involving sampling of first or third instars, newly emerged adults, or adult feeding marks would be more efficient than those requiring sampling for eggs, second instars, pupae, or overwintering adults.
Section IV

IMPACT OF 2,4-D ON CEUTHORHYNCHIDUS HORRIDUS AND THEIR COMPATIBILITY FOR INTEGRATED CONTROL OF CARDUUS THISTLES
Introduction

*Ceuthorhynchidius horridus* (Panzer), a rosette-eweal imported into Canada (Dunn 1978) and the U.S.A. for control of *Carduus* thistles, has become established in Virginia (Kok and Trumble 1979). Basic studies on its host specificity (Kok 1975), laboratory propagation (Trumble and Kok 1978), and life cycles in Italy (Frick 1969) and the U.S.A. (Trumble and Kok 1979b) have provided essential background information for management of this weevil to increase its efficacy in thistle control.

Since 2,4-Dichlorophenoxyacetic acid (2,4-D) is commonly used in Virginia for thistle control (Kates et al. 1979), information on the compatibility of this herbicide with biological control agents is necessary for successful management strategies. Trumble and Kok (1979a) reported that impact of 2,4-D on development and survival of an imported thistle-head weevil, *Rhinocyllus conicus* Froelich. The effects of 2,4-D on *C. horridus* in laboratory and field experiments are presented here.

Materials and Methods

Laboratory Tests

Newly emerged *C. horridus* adults, collected from unsprayed musk (*Carduus nutans* L.) and plumeless thistles (*C. acanthoides* L.) in June, 1978, were separated by sex and caged with bouquets of musk leaves in 0.4 litre plastic containers. After 48 hours, two replicates of 15 males and two replicates of 15 females were treated with sticker plus commercial 2,4-D low volatile amine (LVA) at rates of 0.17, 1.68,
16.8, 84.0, or 147.8 kg/ha. These dosages corresponded to 0.1, 1.0, 10.0, 50.0, and 88.0 times, respectively, the manufacturer's recommended application rate. Controls were sprayed with water plus sticker.

To simulate maximum spray contact in the field, weevils were placed on bare ground in 0.4 litre containers, sprayed using a pressure-calibrated backpack sprayer, and sealed in the containers for 30 minutes. Adults were then returned to cages with musk thistle-leaf bouquets and their feeding monitored every five days for thirty days. Weight change (monitored monthly) and mortality (observed at 24-hour intervals for one week and weekly thereafter) were recorded for 25 weeks. All weevils were maintained in a photoperiod chamber with a light-dark (LD) cycle of LD:16-8. Temperature throughout this test fluctuated between 21°C ± 1°C (day cycle) and 13°C ± 2°C (night cycle).

Log-probit analysis was computer generated using the "Probit Procedure" of SAS (Barr et al. 1976). Acute and chronic toxicity data were normalized with the arcsin transformation and analyzed using the Student-Newman-Keuls test. Differences in adult feeding were analyzed with the Students t-test.

Field Tests

Two thistle sites were selected for field studies. Site #1, located in Montgomery Co., Virginia, near Christiansburg, was treated with 2,4-D at 2.24 kg/ha on May 25, 1977, one week after adults were released. Only plumeless thistles were present at this location. Site #2, located in Pulaski Co., Virginia, at Belspring, was sprayed
with 2,4-D at 1.68 kg/ha on June 27, 1977, approximately two years after initial releases. This field contained both musk and plumeless thistles. The two locations were surveyed for C. horridus larvae and adults each spring in 1978 and 1979 to document survival under field-sprayed conditions.

Results and Discussion

Laboratory Tests

Log probit analysis of the dose-response data after five days indicated that application of 2,4-D at 1.68 kg/ha plus sticker did not cause acute mortality in C. horridus populations. The LC<sub>50</sub> values for males (70.2 kg/ha) and females (61.4 kg/ha) corresponded to 41.8 and 36.6 times, respectively, the recommended dose/ha. Survival of weevils treated with 1.68 kg/ha was not significantly different from controls (Table II). Although application of 2,4-D at 16.8 and 84.0 kg/ha did not significantly increase adult mortality during the first three days of this test, significant differences (P<0.05) in survival were apparent during the remaining 172 days. Treatment of adults with 147.8 kg/ha significantly reduced (P<0.05) adult survival by the third day after herbicide application.

The impact of 2,4-D on C. horridus was primarily acute, as populations treated with 16.8 kg/ha or more declined more rapidly (P<0.05) during the first five days of the test than weevils sprayed with 1.68 kg/ha or less. Differences in rates of decline were not significant between treatments for the remaining 24 weeks.
Table II. Toxicity of Various Concentrations of 2,4-D (LVA) to C. horridus adults.

<table>
<thead>
<tr>
<th>Treatment (kg/ha)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>20</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>98.3 a</td>
<td>93.3 a</td>
</tr>
<tr>
<td>0.17</td>
<td>98.3 a</td>
<td>96.7 a</td>
<td>96.7 a</td>
<td>96.7 a</td>
<td>96.7 a</td>
</tr>
<tr>
<td>1.68</td>
<td>100.0 a</td>
<td>98.3 a</td>
<td>98.3 a</td>
<td>98.3 a</td>
<td>83.0 ab</td>
</tr>
<tr>
<td>16.80</td>
<td>100.0 a</td>
<td>81.7 a</td>
<td>75.0 b</td>
<td>73.3 b</td>
<td>73.3 b</td>
</tr>
<tr>
<td>84.00</td>
<td>100.0 a</td>
<td>85.0 a</td>
<td>70.0 b</td>
<td>66.7 b</td>
<td>66.7 b</td>
</tr>
<tr>
<td>147.84</td>
<td>86.7 a</td>
<td>23.3 b</td>
<td>20.0 c</td>
<td>15.0 c</td>
<td>9.0 c</td>
</tr>
</tbody>
</table>

*Based on 2 replicates of 15♂ and 2 replicates of 15♀; means in columns followed by the same letter are not significantly different at the P < 0.05 level (arcsin transformation, Student-Newman-Keuls test).
Adult vitality, measured by monitoring feeding punctures and weight changes, was not adversely affected by 2,4-D. No significant differences in mean number of feeding punctures/adult (range = 23.86 ± 12.69 - 17.60 ± 9.65) or weight change were found between treatments. However, some common weight fluctuations were observed. A general weight loss of 3-4 mg/weevil between July and the first week in October resulted from reduced feeding (\( \bar{X} = 7.3 \pm 4.5 \) punctures/adult/week) during this period. Increased feeding in the second week in October (\( \bar{X} = 21.1 \pm 12.2 \) punctures/adult/week) corresponded with the termination of summer aestivation of weevils in the field. Weights remained the same or increased slightly until late November when a general decline occurred.

Field Tests

Application of 2,4-D to thistle-infested fields did not prevent survival and reproduction of \( C. \ horridus \) populations. In spite of the death of host plants and the long-term exposure to herbicide residue, larvae and adults were recovered during the spring of 1978 from both test locations. Few thistles and no weevils were found at site 1 during the spring of 1979, because thistles at this site were destroyed in the summer and fall of 1978 by repeated applications of 2,4-D at 2.24 kg/ha and kerosene. However, in Pulaski Co., adults had increased to over 250% of initial releases (Kok and Trumble 1979) by spring of 1978, and were present in large numbers in the spring of 1979 despite an additional 2,4-D application at 1.68 kg/ha in June 1978.
Results of these tests indicate that 2,4-D (LVA) does not adversely affect the survival of *C. horridus*, and can be compatibly used with the weevil in an integrated control program for *Carduus* thistles. Trumble and Kok (1979a) reported that spring application of 2,4-D on musk thistles in the late-bud to early-bloom stages of the primary blooms did not inhibit *Rhinocyllus conicus* larval development, but did prevent production of viable seed. At this stage of plant development, *C. horridus* occurs primarily in the pupal or adult stages (Trumble and Kok 1979b). Since the pupae develop underground (Kok et al. 1975), and 2,4-D is absorbed on soil colloids, they are not likely to be seriously affected by normal herbicide rates and application techniques. Because laboratory tests indicated that survival and vitality were not affected by treatment with 2,4-D at recommended dosages, and field trials revealed that adult survival and reproduction could occur in sprayed fields, 2,4-D applications may be used to reduce thistle densities without destruction of *C. horridus* populations.
Section V

COMPATIBILITY OF RHINOCYLLUS CONICUS LARVAE (COLEOPTERA: CURCULIONIDAE) AND 2,4-D FOR CONTROL OF CARDUUS NUTANS
Introduction

*Carduus nutans* L. (musk thistle), a noxious European import, has been the target of extensive control efforts in the United States and Canada. Biological (Harris and Zwölfer 1971, Surles et al. 1974, Puttler et al. 1978) as well as chemical and mechanical procedures (Kates 1968, McCarty and Hatting 1975) have been used to regulate this weed. Reduction of musk thistle seed production by the thistle-head weevil, *Rhinocyllus conicus* Froelich, was significant (Rees 1977, Surles and Kok 1978), as well-timed applications of herbicides (McCarty and Hatting 1975). However, these studies did not relate the respective stages of weevil and plant development to the time of herbicide application. Consequently, the objective of this study was to determine compatibility of the herbicide 2,4-D and the biocontrol agent *R. conicus* in a musk thistle management program.

Materials and Methods

Ovipositing *R. conicus* were caged on the primary (terminal) heads of musk thistle at a study site in Giles Co., Virginia. The weevils and all but five eggs/head were removed after 48 h. Light cotton sacks, used to confine weevils to heads, were retained to reduce parasite and predator influence, and to prevent additional oviposition and seed loss while the heads remained on the plants. Twelve replicated sprays of commercial 2,4-D (LVA) at 1.68 kg/ha (plus sticker) were conducted at 0 (within 48 h), 1, 2 and 3 weeks post-oviposition on randomly selected thistles. The cotton sacks
were removed before spraying and replaced after herbicide application; entire plants were sprayed. Control plants were not sprayed because counts after the first spray indicated that no eggs were dislodged. Herbicide application at 0 weeks (June 1, 1978) coincided with current extension recommendations \(^1\) for musk thistle control in Giles Co., Virginia (elevation 850 m). Terminal heads were in the early-bud stage, and plants were approximately 40 cm in height upon initiation.

Following senescence, heads were excised and brought to the laboratory to count and weigh newly emergent weevils. After six weeks heads were dissected and larval, pupal or adult cadavers, and living adults were counted. Seeds not physically damaged by \(R.\) \textit{conicus} were placed on moistened filter paper, using the technique described by Surles and Kok (1978), and observed for seven days to quantify germination.

\textbf{Results and Discussion}

Mortalities of larvae developing from plants sprayed 1-3 weeks after oviposition, and from untreated plants, were significantly lower than mortalities from plants sprayed at 0 weeks (within 48 hours of oviposition) (Table III). The latter failed to support larval development beyond second instar. Developmental times of \(R.\) \textit{conicus} were similar for all treatments producing adults.

Table III. Mortality and Mean Developmental Times of R. conicus, and Per Cent Germination of Undamaged Seeds From Musk Thistles Treated at Weekly Intervals with 2,4-D\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage of plant</th>
<th>% Mortality\textsuperscript{2}</th>
<th>No. weeks to adult emergence</th>
<th>% Germination of undamaged seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>9.1 a</td>
<td>11.9 a</td>
<td>71.8 c</td>
</tr>
<tr>
<td>0 week spray</td>
<td>bud</td>
<td>100.0 b</td>
<td>0\textsuperscript{3}</td>
<td>0 a</td>
</tr>
<tr>
<td>1 week spray</td>
<td>late bud</td>
<td>12.5 a</td>
<td>11.3 a</td>
<td>0 a</td>
</tr>
<tr>
<td>2 week spray</td>
<td>early bloom</td>
<td>19.3 a</td>
<td>11.8 a</td>
<td>0 a</td>
</tr>
<tr>
<td>3 week spray</td>
<td>full bloom</td>
<td>11.1 a</td>
<td>11.5 a</td>
<td>9.9 b</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Based on 12 replications of 5 eggs/head each; 2,4-D applied at 1.68 kg/ha. Means followed by the same letter in each column do not differ significantly at the P < 0.05 level (Student-Newman-Keuls test).

\textsuperscript{2} Excluding egg stage and first instars not entering the head.

\textsuperscript{3} No adult emergence, development to second instar only.
No viable seeds were produced by thistles sprayed from 0 to 2 weeks after oviposition. The primary heads were in bud stage for the 0 to 1 week sprays and in late-bud to early-bloom at the 2 week treatment. Primary heads were in full bloom when the plants were treated 3 weeks after oviposition, with average height exceeding 80 cm. The increased time available for development and the reduced effect of 2,4-D allowed some seeds from the 3 week treatment to reach a viable state. Mean per cent germination of seeds undamaged by R. conicus (approximately 10%) was significantly lower than the control (71.8%).

Although reduction in seed viability occurred from spraying infested plants with primary heads in full bloom, application of 2,4-D during bud stage was more effective in inhibiting seed production and killing the thistles. These results are similar to those of McCarty and Hatting (1975) who found that application of 2,4-D ester at 2.24 kg/ha during the early-bloom stage was more effective in reducing viable seed than treatment of plants in full-bloom. In my tests, absence of viable seeds from plants sprayed in the late-bud to early-bloom stage could be attributed to reduction in seed size, number, and viability from R. conicus infested heads (Rees 1977, Surles and Kok 1978), as well as to herbicidal effects. Germination in control seeds was similar to that found by Surles and Kok (1978).

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2."Bud" and "Bloom" stages: as used in McCarty and Hatting (1975).
Herbicide treatments did not cause any significant differences in weights of emergent ($\bar{X} = 154.2$ mg) weevils. However, those insects which failed to emerge from thistle heads averaged 31.6 mg less, appeared weak, and may not have been capable of survival in the field. Nonemergent populations were small, ranging from 6-18% per treatment.

Rees (1977) stated that the effects of herbicides on *R. conicus* survival varied with weevil density; as the larval population/head increased, survival decreased. My field observations have shown that proper timing of herbicide application can reduce weevil larval density/head. Treatment during late-bud to early-bloom stage of the primary head interrupts oviposition by the weevils due to the death of the plant, causing fewer eggs to be laid on treated plants. Since treated plants become less attractive for feeding and oviposition, weevil movement to healthy unsprayed plants could occur. This could improve egg distribution and increase biological pressure against thistles in areas where spraying is uneconomical or unfeasible. Therefore, by spraying musk thistles with 2,4-D in the late-bud to early-bloom stages of the primary head, seed germination can be prevented without the destruction of *R. conicus* populations. Integration of herbicidal and biological control of thistles can be implemented by relating the application of herbicides to insect and plant developmental stages.
Section VI

INTEGRATION OF RHINOCYLLUS CONICUS ADULTS AND 2,4-D FOR CARDOUS THISTLE CONTROL
Introduction

Interest in developing biological and integrated management techniques for *Carduus* thistles has increased substantially during the past ten years (Kok and Surles 1975, McCarty and Hatting 1975, Dunn 1978). Searches for biological control agents for *Carduus* thistles in many European countries (Zwölfer 1965, Boldt and Campobasso 1978, Boldt 1978) have led to the screening (Zwölfer 1967, Kok 1975, Dunn and Rizza 1976), release and establishment of a variety of thistle-feeding insects in North America (Dunn 1978, Goeden and Ricker 1978, Kok and Trumble 1979, Puttler et al. 1978).

Despite increased interest in biological approaches to thistle control, current control practices still rely heavily on the use of herbicides, especially 2,4-Dichlorophenoxyacetic acid (2,4-D) (Kates et al. 1979). Thus, the effects of pesticides on beneficial insects attacking *Carduus* thistles must be evaluated prior to development of an integrated approach to pasture management for these noxious weeds. Experimentally-based information permitting development of management strategies has only recently become available. Rees (1977) stated that the effects of herbicides on survival of a thistle-head weevil, *Rhinocyllus conicus* Froelich, varied with larval density; as larval population/thistle bloom increased, survival decreased. Trumble and Kok (1979a) related the respective stages of weevil and plant development to timing of herbicide application. They showed that treatment of musk thistle (*Carduus nutans* L.) with 2,4-D in the late-bud to early-bloom stage of the primary bloom prevented formation
of viable seed without adversely affecting \textit{R. conicus} larval development. Based on the impact of 2,4-D low volatile amine (LVA) on \textit{R. conicus} adults in laboratory and field experiments, the potential for integration of the herbicide and biocontrol agent was examined.

\textbf{Materials and Methods}

\textbf{Herbicide Treatment of \textit{R. conicus} Prior to Overwintering}

\textit{R. conicus} adults emerging from musk thistle blooms collected in Pulaski Co., Virginia, in July, 1978, were separated by sex and caged with bouquets of thistle leaves. After two weeks, 2 replicates of \textit{20} and 2 replicates of \textit{20'} were treated with adjuvent (sticker = Nufilm \textsuperscript{17}) plus commercial 2,4-D (LVA) at rates of 0.17, 1.68, 16.80, 84.00, or 147.84 kg/ha. These rates corresponded to 0.1, 1.0, 10.0, 50.0, or 88.0 times, respectively, the manufacturer's recommended application rate. Controls were sprayed with water and adjuvent only.

To simulate maximum initial spray contact in the field, weevils were placed on compacted earth in 0.4 litre containers, sprayed using a pressure-calibrated backpack sprayer, and confined on the treated surface for 30 minutes. Adults were then returned to cages and monitored weekly for mortality. All weevils were maintained in photoperiod chambers with a light-dark (LD) cycle of LD:16–8 until October, when they were switched to LD:8–16 as natural photoperiods decreased. Temperatures throughout this test fluctuated between 21\textdegree C \pm 1\textdegree C (day cycle) and 13\textdegree C \pm 2\textdegree C (night cycle).
After 24 weeks, no less than 8 pairs of adults (♀♂) were randomly selected from weevils remaining in all but the 147.84 kg/ha treatment and caged with primary blooms of musk thistle to determine if oviposition would occur. Treatments at 147.84 kg/ha resulted in too few survivors for incorporation into this experiment. Thistle bloom stems were immersed in water to retain freshness and suitability for oviposition; blooms were replaced at least every 6 days. Eggs were removed, counted, and placed on moistened filter paper each time blooms were replaced. Viability was determined by the occurrence of egg hatch. Adults were maintained at LD:16–8 and 21°C ± 1°C throughout this oviposition study.

Herbicide Treatment of R. conicus After Overwintering

Adult weevils emerging from overwintering sites were collected from musk thistle in Pulaski Co., Virginia, in early May, 1979. Three replicates of 20♀ and 3 replicates of 20♂ were treated using the same dosages and techniques as adults in the previous study. Temperature and photoperiod were maintained at 21°C ± 1°C and LD:16–8, respectively. Adult mortality was monitored daily for 14 days.

To determine if fecundity was affected by 2,4-D applications, 12 replicates of 2♀ plus 2♂ from each treatment were caged in 0.2 litre containers with musk thistle blooms. Eggs were removed and counted at 3-day intervals for 21 days and blooms were replaced at least every 6 days. Egg viability was monitored as in the previous experiments. Adult mortality was recorded for each sample date.
Herbicide Treatment with \textit{R. conicus} on Thistle Rosettes

Additional experiments were designed to document the survival and fecundity of adults on musk thistle rosettes because \textit{R. conicus} living on thistles do not receive identical herbicide doses, and the weevils would be exposed to pesticide residues through contact and ingestion. Six replicates of $10^9$ and $10^9$ were placed on musk thistles rosettes, allowed to acclimate for 5 minutes, then sprayed with sticker plus 2,4-D (LVA) at 1.68 kg/ha. Also, 6 replicates of $10^9$ and $10^9$ were released on musk thistle that had been previously sprayed with the herbicide at 1.68 kg/ha and allowed to dry. Controls were treated with water plus sticker only. The rosettes used in this study were of comparable diameter ($\overline{x} = 26.4 \pm 2.1$ cm). Weevils were confined on the plants for 1 week, at which time all herbicide treated rosettes had died. Adults were then removed, placed in 0.2 litre containers with musk thistle blooms, and monitored for fecundity and egg viability as in the previous experiment.

Field Application of Herbicide to \textit{R. conicus} Infested Thistles

Two thistle sites were selected for field studies. Site #1, located in Montgomery Co., Virginia, was treated with 2,4-D at 2.24 kg/ha on May 25, 1977, 15 days after \textit{R. conicus} adults had been released. Only plumeless thistle (\textit{Carduus acaanthoides} L.) was present at this location. Site #2, located in Pulaski Co., Virginia, was sprayed with 2,4-D at 1.68 kg/ha on June 27, 1977, at least 3 years after \textit{R. conicus} became established. This field contained both musk and plumeless thistle. These locations were surveyed for \textit{R. conicus}
adults each spring in 1978 and 1979 to document survival under field-sprayed conditions.

**Statistical Analysis**

Log probit analysis was computer generated using the "Probit Procedure" of SAS (Barr et al 1976). Acute and chronic toxicity data, normalized with the arcsin transformation, and differences in fecundity between treatments were analyzed using the Student-Newman-Keuls test.

**Results and Discussion**

**Herbicide Treatment of *R. conicus* Prior to Overwintering**

Log probit graphs of the dose-response data after 14 days indicated that application of 2,4-D at 1.68 kg/ha plus sticker did not cause mortality in *R. conicus* populations prior to overwintering. The LC$_{50}$ values for males (78.6 kg/ha) and females (66.8 kg/ha) corresponded to 46.8 and 39.8 times, respectively, the recommended dose/ha. Survival of weevils treated with up to 10X the recommended dose/ha (16.8 kg/ha) was not significantly different from controls (Table IV). Although application of 2,4-D at 84.0 kg/ha did not cause adult mortality during the first week of this test, significant reductions ($P < 0.05$) in survival were apparent during the remaining 24 weeks. Treatment of adults with 147.84 kg/ha significantly reduced survival throughout this study ($P < 0.05$). However, herbicidal effects on oviposition and egg viability were not observed; all treatments contained females ($n = 4-6$) which produced viable eggs.
Table IV. Survival of *R. conicus* Adults Treated Before Overwintering With Various Concentrations of 2,4-D (LVA).

<table>
<thead>
<tr>
<th>Treatment (kg/ha)</th>
<th>% survival post-treatment (weeks)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0 (control)</td>
<td>100.0 a</td>
</tr>
<tr>
<td>0.17</td>
<td>100.0 a</td>
</tr>
<tr>
<td>1.68</td>
<td>100.0 a</td>
</tr>
<tr>
<td>16.80</td>
<td>98.7 a</td>
</tr>
<tr>
<td>84.00</td>
<td>100.0 a</td>
</tr>
<tr>
<td>147.84</td>
<td>72.5 b</td>
</tr>
</tbody>
</table>

*Based on 2 replicates of 20% and 2 replicates of 20% treatment; means in columns followed by the same letter do not differ at the P < 0.05 level (Student-Newman-Keuls test, arcsin transformation).
The impact of 2,4-D on *R. conicus* was primarily acute, as populations treated with 16.8 kg/ha or more declined more rapidly than weevils sprayed with 1.68 kg/ha or less (*P < 0.05*) during the first 5 weeks after herbicide application (Table V). Differences in rates of decline were not significant between treatments for the remaining 19 weeks.

**Herbicide Treatment of *R. conicus* After Overwintering**

LC\(_{50}\) values after 14 days for males (117.1 kg/ha) and females (126.6 kg/ha) corresponded to 69.7 and 75.4 times, respectively, the recommended dosage of 1.68 kg/ha. These values are slightly higher than corresponding values obtained from treatment of weevils prior to overwintering, and mortality data (Table VI) show that 2,4-D has less impact on survival of overwintered adults. Application of 2,4-D at 84.00 kg/ha to overwintered adults initially caused significant differences (*P < 0.05*) in survival, but percentage survival beyond 3 days was not significantly different from controls. Sprays of 2,4-D at 147.84 kg/ha significantly reduced survival throughout this test (*P < 0.05*).

Fecundity of overwintered weevils was reduced when treated with 2,4-D at rates of 1.68, 16.8 and 147.84 kg/ha (Table VII). However, in each of these treatments several males died within 3 days, and the corresponding females produced few or no eggs. If these females are not included in the analysis, no significant differences in mean numbers of eggs/female can be detected. In addition, if only ovipositing females are considered (Table VII), the mean numbers of
Table V. Rate of Decline of *R. conicus* Populations Treated Before Overwintering With Various Rates of 2,4-D (LVA).

<table>
<thead>
<tr>
<th>Treatment (kg/ha)</th>
<th>% Mortality/Week*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5 weeks</td>
</tr>
<tr>
<td>0 (control)</td>
<td>0.25 a</td>
</tr>
<tr>
<td>0.17</td>
<td>0.0 a</td>
</tr>
<tr>
<td>1.68</td>
<td>0.25 a</td>
</tr>
<tr>
<td>16.80</td>
<td>2.25 b</td>
</tr>
<tr>
<td>84.00</td>
<td>6.00 c</td>
</tr>
<tr>
<td>147.84</td>
<td>16.50 d</td>
</tr>
</tbody>
</table>

*Based on 2 replicates of 20♀ and 2 replicates of 20♂/treatment; means in columns followed by the same letter do not differ at the P < 0.05 level (arcsin transformation, Student-Newman-Keuls test).
Table VI. Survival of Overwintered *R. conicus* Adults Treated With Various Concentrations of 2,4-D (LVA).

<table>
<thead>
<tr>
<th>Treatment (kg/ha)</th>
<th>% survival post-treatment (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0 (control)</td>
<td>99.2 a</td>
</tr>
<tr>
<td>0.17</td>
<td>100.0 a</td>
</tr>
<tr>
<td>1.68</td>
<td>100.0 a</td>
</tr>
<tr>
<td>16.80</td>
<td>99.2 a</td>
</tr>
<tr>
<td>84.00</td>
<td>90.8 b</td>
</tr>
<tr>
<td>147.84</td>
<td>84.2 b</td>
</tr>
</tbody>
</table>

*Based on 3 replicates of 20¢ and 3 replicates of 20c/treatment; means in columns followed by the same letter do not differ at the P < 0.05 level (arcsin transformation, Student-Newman-Keuls test).
Table VII. Impact of Various Concentrations of 2,4-D (LVA) on Fecundity of Overwintered R. conicus.

<table>
<thead>
<tr>
<th>Treatment (kg/ha)</th>
<th>$\bar{x}$ eggs/p 21 days $^1$</th>
<th>$\bar{x}$ eggs/p 3 day interval $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>37.58 a</td>
<td>6.15 a</td>
</tr>
<tr>
<td>0.17</td>
<td>44.54 a</td>
<td>7.08 a</td>
</tr>
<tr>
<td>1.68</td>
<td>27.23 b</td>
<td>5.51 a</td>
</tr>
<tr>
<td>16.80</td>
<td>26.68 b</td>
<td>5.64 a</td>
</tr>
<tr>
<td>84.00</td>
<td>37.83 a</td>
<td>7.60 a</td>
</tr>
<tr>
<td>147.84</td>
<td>27.61 b</td>
<td>5.44 a</td>
</tr>
</tbody>
</table>

$^1$ Based on 12 replicates of 2 /treatment.

$^2$ Based on ovipositing ñs only (n = 10-12 replicates of 2ñ/treatment); means in columns followed by the same letter do not differ at the P < 0.05 level (Student-Newman-Keuls test).
eggs/female/3 day sampling interval are not significantly different between treatments. All ovipositing females in this study produced viable eggs regardless of the herbicide dosage administered.

Herbicide Treatment of R. conicus on Thistle Rosettes

Exposure of R. conicus adults on musk thistle rosettes to 2,4-D (LVA) sprays and/or residue did not inhibit survival or reproduction. Survival at 2 weeks post-treatment was not significantly different from controls (range = 79.2% - 83.7%), and LC$_{50}$ values could not be calculated. The impact of 2,4-D on oviposition as measured by numbers of eggs/female/21 days (range = 28.2 - 31.4) and numbers of eggs/ovipositing female/3 day period (range = 6.2 - 6.8) was not significant. With the exception of the numbers of eggs/female/21 days, these values are not significantly different from comparable data in the post-overwintering study. The slightly lower values for numbers of eggs/female/21 days for adults treated on rosettes versus controls for overwintered weevils may be attributed to the lack of oviposition by 2 females in each residue treatment. If these weevils are not included in the analysis, no significant differences are apparent. As in the previous oviposition tests, all ovipositing females produced viable eggs.

Field Application of Herbicide to R. conicus Infested Thistles

Application of 2,4-D to thistle-infested fields did not prevent survival and reproduction of R. conicus. In spite of the death of host plants and exposure of adults to herbicide residue, larvae and
adults were recovered during the spring of 1978 from both test locations. Few thistles and no weevils were found at site #1 in spring of 1979 because the thistle population was destroyed during the summer and fall of 1978 by repeated applications of 2,4-D and kerosene. However, adults and larvae were abundant in Pulaski Co., Virginia, in spring 1979, despite an additional 2,4-D treatment at 1.68 kg/ha in June, 1978.

**Conclusions**

Results of these tests indicate that 2,4-D does not adversely affect the survival of *R. conicus*, and can be compatibly used with the weevil in an integrated control program for *Carduus* thistles. 2,4-D treatment of musk thistle in the late-bud to early-bloom stage of the primary bloom was not detrimental to the larvae (Trumble and Kok 1979a), and would not prevent survival or reproduction of adult *R. conicus*. Although 2,4-D treatment of rosettes does not directly affect *R. conicus*, herbicide application may seriously impair the efficacy of rosette-feeding insects such as *Ceuthorhynchidius horridus* (Panzer). *C. horridus* occurs primarily in the larval stage in the U.S.A. when thistles are in the rosette stage (Trumble and Kok 1979b). Herbicide treatment in the late-bud to early-bloom stage of the primary bloom of musk thistle would coincide with the pupal and adult stages of *C. horridus*. The pupal stage develops underground, and the adult stage is not adversely affected by 2,4-D at 1.68 kg/ha.
Section VII

SUMMARY
The data presented in this study indicate that herbicide application can be coordinated with thistle development to reduce the undesirable effects of 2,4-D on both R. conicus and C. horridus. Thus, farmers and agriculturists can apply 2,4-D to economic infestations of thistles and achieve immediate results, without inhibiting the initial increase in biological control agent populations.

I propose several recommendations that are suitable for distribution to pasture owners as part of an IPM program. The recommendations presented below integrate the biological control agents with the primary chemical control, and represent a synthesis of all the investigations that I have conducted and of related work of which I am aware.

A. Spring: 1) Apply 2,4-D when musk thistles are just beginning to bolt (average plant height = 5-15 cm, tallest plants = 20 cm.)

2) Apply 2,4-D when the terminal bloom of musk thistle is in the late-bud to early-bloom stage of development (average plant height = 30-35 cm, tallest plants = 45 cm).

B. Fall: Apply 2,4-D after seeds have germinated, but before mid-October.

Spring application of 2,4-D when musk thistle is just beginning to bolt is the most advantageous time for herbicide treatment. Thistles at this stage of development are rapidly growing and the herbicide is readily translocated to sensitive tissues, resulting in death of the
treated plants. Further, the biological control agents are not seriously affected by 2,4-D at this time. *R. conicus* adults are not only tolerant of 2,4-D (*LC*<sub>50</sub> values average 120 kg/ha) but have not yet begun to oviposit because the plants have not reached the budding stage. *C. horridus* occurs in the pupal stage when the plants are beginning to bolt and field trials indicate herbicide treatment during this stage of development does not adversely affect weevil survival, reproduction or population increase.

2,4-D can also be applied when the terminal bloom of musk thistle is in the late-bud to early-bloom stage. Treatment at this time causes death of the host plants, but does not prevent survival of *R. conicus* populations. Although most *C. horridus* have become adults by this stage of plant development, the weevils are tolerant to 2,4-D application at the recommended rates (*LC*<sub>50</sub> values average 68 kg/ha). Field observations also indicate that *R. conicus* and *C. horridus* adults treated while on thistles will move to unsprayed plants. This may increase the biological pressure on *Carduus* species in areas where herbicide use is unfeasible or uneconomical.

Fall applications of herbicides can also be used compatibly with biological control agents for thistle control, although timing sprays during the fall is more difficult than in the spring. Treatments should be late enough in the season for most thistle seeds to have germinated, but early enough that the herbicide can be applied while the plants are still growing. In southwestern Virginia, treatment during early to mid-October generally assures
enough warm weather for 2,4-D to effectively reduce thistle populations, and for C. horridus adults to move to untreated plants before the onset of cold weather. Herbicide treatments should be adjusted to achieve similar results at other locations.

Thus, 2,4-D applications can be manipulated to have a minimal impact on the complex of biological control agents present. By timing herbicide treatments to manage thistle populations without adversely affecting the biological control agents, use of herbicides may be reduced without increasing thistle densities. Additional information on pasture management is necessary to complete an IPM package on thistle control.
LITERATURE CITED


_________________________. 1979a. Compatibility of *Rhinocyllus conicus* (Coleoptera:Curculionidae) and 2,4-D for musk thistle control. Environ. Entomol. 8:421-2.


APPENDIX
Appendix A - A Bibliography of *Ceuthorhynchidius horridus*


79. __________. 1980a. Impact of 2,4-D on *Ceuthorhynchidius horridus* (Coleoptera:Curculionidae) and their compatibility for integrated control of *Carduus* thistles. *Weed Res.* 20: (in press).


* These references not seen by author.
APPENDIX B

Table VIII. Preliminary Results of Stratified-Random Transect Sampling of _C. nutans_ for _R. conicus_ by hand and with D-Vac: Rosettes Included\(^1\).

<table>
<thead>
<tr>
<th>Date</th>
<th>Technique</th>
<th>plant height x adults</th>
<th>no. eggs x adults</th>
<th>plant height x eggs</th>
<th>no. eggs x adults</th>
<th>plant height x heads</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-V</td>
<td>D-Vac</td>
<td>0.89</td>
<td>----</td>
<td>----</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>10-V</td>
<td>hand</td>
<td>0.68</td>
<td>----</td>
<td>----</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>17-V</td>
<td>D-Vac</td>
<td>0.65</td>
<td>0.69</td>
<td>0.81</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>17-V</td>
<td>hand</td>
<td>0.52</td>
<td>0.68</td>
<td>0.69</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>24-V</td>
<td>D-Vac</td>
<td>0.77</td>
<td>0.81</td>
<td>0.81</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>24-V</td>
<td>hand</td>
<td>0.59</td>
<td>0.68</td>
<td>0.63</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>31-V</td>
<td>D-Vac</td>
<td>0.45</td>
<td>0.77</td>
<td>0.45</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>31-V</td>
<td>hand</td>
<td>0.52</td>
<td>0.78</td>
<td>0.66</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Sample size no less than 18; samples taken in Giles Co., Virginia.

\(^2\) No relationship measurable (no eggs present).
APPENDIX B (cont.)

Table IX. Preliminary Results of Arbitrary Sampling of *C. nutans* for *R. conicus* by Hand and With D-Vac: Rosettes Included$^1$.

<table>
<thead>
<tr>
<th>Date</th>
<th>Technique</th>
<th>plant height x adults</th>
<th>plant height x eggs</th>
<th>no. eggs x adults</th>
<th>plant height x no. heads</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-V</td>
<td>D-Vac</td>
<td>0.79</td>
<td>0.77</td>
<td>0.87</td>
<td>0.67$^2$</td>
</tr>
<tr>
<td>16-V</td>
<td>hand</td>
<td>0.77</td>
<td>0.78</td>
<td>0.93</td>
<td>----</td>
</tr>
<tr>
<td>23-V</td>
<td>D-Vac</td>
<td>0.85</td>
<td>0.89</td>
<td>0.92</td>
<td>0.89</td>
</tr>
<tr>
<td>23-V</td>
<td>hand</td>
<td>0.68</td>
<td>0.64</td>
<td>0.89</td>
<td>0.67</td>
</tr>
<tr>
<td>30-V</td>
<td>D-Vac</td>
<td>0.66</td>
<td>0.80</td>
<td>0.69</td>
<td>0.82</td>
</tr>
<tr>
<td>30-V</td>
<td>hand</td>
<td>0.37</td>
<td>0.72</td>
<td>0.33</td>
<td>0.75</td>
</tr>
</tbody>
</table>

$^1$Based on samples of 50 plants in Pulaski Co., Virginia; 1979.

$^2$No relationship measurable.
APPENDIX B (cont.)

Table X. Preliminary Results of Stratified-Random Transect Sampling of C. nutans for R. conicus by Hand and With D-Vac: Rosettes Not Included.¹

<table>
<thead>
<tr>
<th>Date</th>
<th>Technique</th>
<th>plant height x adults</th>
<th>plant height x eggs</th>
<th>no. eggs x adults</th>
<th>plant height x heads</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-V</td>
<td>D-Vac</td>
<td>0.93</td>
<td>----²</td>
<td>NS³</td>
<td>----</td>
</tr>
<tr>
<td>10-V</td>
<td>hand</td>
<td>0.41</td>
<td>----</td>
<td>0.51</td>
<td>----</td>
</tr>
<tr>
<td>17-V</td>
<td>D-Vac</td>
<td>0.51</td>
<td>0.63</td>
<td>0.40</td>
<td>0.74</td>
</tr>
<tr>
<td>17-V</td>
<td>hand</td>
<td>0.55</td>
<td>0.83</td>
<td>0.58</td>
<td>0.77</td>
</tr>
<tr>
<td>24-V</td>
<td>D-Vac</td>
<td>0.66</td>
<td>0.79</td>
<td>0.56</td>
<td>0.70</td>
</tr>
<tr>
<td>24-V</td>
<td>hand</td>
<td>0.42</td>
<td>NS</td>
<td>0.45</td>
<td>0.47</td>
</tr>
<tr>
<td>31-V</td>
<td>D-Vac</td>
<td>NS</td>
<td>0.82</td>
<td>0.50</td>
<td>NS</td>
</tr>
<tr>
<td>31-V</td>
<td>hand</td>
<td>NS</td>
<td>0.58</td>
<td>0.70</td>
<td>0.46</td>
</tr>
</tbody>
</table>

¹Sample size no less than 25: samples taken in Giles Co., Virginia, 1979.

²No relationship measurable: no eggs.

³NS = relationship not significant at the 95% level.
APPENDIX B (Cont.)

Table XI. Preliminary Results of Arbitrary Sampling of _C. nutans_ for _R. conicus_ by Hand and With D-Vac: Rosettes Not Included.¹

<table>
<thead>
<tr>
<th>Date</th>
<th>Technique</th>
<th>plant height x adults</th>
<th>plant heights x eggs</th>
<th>no. eggs x adults</th>
<th>plant height x heads</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-V</td>
<td>D-Vac</td>
<td>0.75</td>
<td>0.74</td>
<td>0.44</td>
<td>0.85</td>
</tr>
<tr>
<td>16-V</td>
<td>hand</td>
<td>0.80</td>
<td>0.80</td>
<td>NS²</td>
<td>0.95</td>
</tr>
<tr>
<td>23-V</td>
<td>D-Vac</td>
<td>0.82</td>
<td>0.88</td>
<td>0.66</td>
<td>0.90</td>
</tr>
<tr>
<td>23-V</td>
<td>hand</td>
<td>NS</td>
<td>0.66</td>
<td>0.83</td>
<td>0.47</td>
</tr>
<tr>
<td>30-V</td>
<td>D-Vac</td>
<td>0.64</td>
<td>0.79</td>
<td>0.81</td>
<td>0.66</td>
</tr>
<tr>
<td>30-V</td>
<td>hand</td>
<td>0.36</td>
<td>0.73</td>
<td>0.77</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹Based on samples of 50 plants in Pulaski Co., Virginia, 1979.

²NS = relationship not significant at the 95% level.
VITA

The author was born on May 24, 1952, in Annapolis, Maryland. After graduation from Marple-Newton Senior High School in June of 1970, he entered the University of Delaware the following September. During his last two years he worked as a laboratory technician on two projects: control of Phorid flies in mushrooms and a biocontrol program for Ostrina nubilalis (Hub.) using Trichogramma nubilala Eartle and Davis. The degree of Bachelor of Science of Agriculture was awarded to him in May, 1974, after he had completed the prescribed course of instruction in Entomology and Applied Ecology. Immediately following graduation he worked as a laboratory technician and as a Plant Pest Inspector at Virginia Polytechnic Institute and State University until December of 1974.

Mr. Trumble entered V.P.I. and S.U. in January, 1975, as a research assistant in the Entomology Department. From June of 1975 until June of 1977 he was a Fellow under the McCormick Scholarship. He was awarded the Master of Science degree in Entomology in Spring of 1977 for nutritional and biological studies on Ceuthorhynchidius horridus (Panzer). In the Fall of 1977 he received the Outstanding Master's Candidate Award from the Eastern Branch of the Entomological Society of America. He is a member of Phi Kappa Phi, Phi Sigma, Gamma Sigma Delta and Sigma Xi.
INTEGRATED CONTROL OF CARDUUS THISTLES AND ECOLOGICAL STUDIES ON RHINOCYLLUS CONICUS
FROELICH AND CEUTHORHYNCHIDIUS HORRIDUS (PANZER)

by

John Thomas Trumble

(ABSTRACT)

A biological and integrated control program for Carduus thistles was developed using the biological control agents Rhinocyllus conicus, Froelich and Ceuthorhynchidius horridus (Panzer) and the herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D).

Field studies on the development of Ceuthorhynchidius horridus (Panzer) on Carduus thistles in Virginia between 1975-1978 showed that the weevil has one generation annually. Oviposition occurred from mid-December until early April, and larvae occurred in rosettes from late December through late May. Teneral adults, which appeared in mid-May through June, underwent an aestival diapause during most of July through September. Adult reappearance in late September coincided with an increase in feeding. Although adult feeding marks, teneral adults and first and third instar larvae were easily found in the field, detection of eggs, second instars or overwintering adults was difficult and time consuming.

Acute and chronic effects of spring application of 2,4-D (LVA) on adult C. horridus were examined. LC$_{50}$ values for males (70.2 kg/ha) and females (61.4 kg/ha) corresponded to 41.7 and 36.6 times, respectively, the recommended application rate of 1.68 kg/ha.
Treatment with 1.68 kg/ha did not affect adult survival, but increased dosages (16.8-147.8 kg/ha) caused significantly greater mortality. Adult vitality, measured by number of feeding marks/weevil and weight change/time, was unaffected by the herbicide. Field application of herbicides did not prevent survival, reproduction, or population increase of *C. horridus*.

Herbicidal effect on larval *R. conicus* was studied by examining the mortality, emergence rates and weights of weevils developing from plants treated with 2,4-D (LVA). Infested heads, obtained by caging ovipositing *R. conicus* on primary heads of musk thistle (*Carduus nutans L.*) (resembles *C. thoermerti* Weinmann), were treated with 2,4-D at 1.68 kg/ha 0–3 weeks after oviposition. Blooms treated immediately following oviposition failed to support larval development beyond the second instar. Developmental times and weights of weevils that emerged from blooms sprayed at 1, 2, and 3 weeks were not significantly different from controls. Plants sprayed up to 2 weeks after oviposition (late-bud to early-bloom) did not produce viable seeds. Treatments at 3 weeks after oviposition (full-bloom) allowed 10% germination of seeds not damaged by *R. conicus* in primary heads, and plants survived to produce additional heads.

Acute and chronic effects of the herbicide 2,4-D on adult *R. conicus* were also examined. *LC*$_{50}$ values for adults prior to overwintering (males 78.6 kg/ha; females 61.0 kg/ha) were lower than those for overwintered weevils (males 117.1 kg/ha; females 126.6 kg/ha), but were still at least 40 times the recommended application
rate of 1.68 kg/ha. Survival was not significantly affected by
direct application of 2,4-D at 1.68 kg/ha plus sticker or by
exposure to herbicide sprays and residue while on musk thistle
rosettes. Mean egg production/ovipositing female/3 day period was
not significantly different (range = 5.44 - 7.60), regardless of
the 2,4-D dosage applied (range = 0.0 - 147.84 kg/ha); all
ovipositing weevils produced viable eggs. Field treatment with
up to 2.24 kg/ha of 2,4-D resulted in death of host plants, but
did not prevent survival or reproduction of R. conicus populations.