CEUTHORRHYNCHIDIUS HORRIDUS PANZER (COLEOPTERA: CURCULIONIDAE) - THE HOST SPECIFICITY OF THE FIRST INSTAR, NOTES ON THE INSECT'S BIOLOGY, AND METHODS OF ADULT AESTIVAL DIAPAUSE TERMINATION

by

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INTRODUCTION

Ceuthorrhynchidius horridus Panzer is a phytophagous weevil which has been found associated with thistles of the subtribe Carduinae (Compositae: Cynareae). Many species within Carduinae (Carduus nutans L., Carduus acanthoides L., Cirsium arvense (L.) Scop., Cirsium vulgare (Savi) Ten., Silybum marianum (L) Gaertn., Onopordum acanthium L., and others) are becoming an ever increasing menace to range and pasture lands. Much of these lands are usually of low economic value and are inaccessible for regular control measures. Since many of these plant species have been introduced accidentally from Eurasia without their complement of natural enemies, checking their rapid spread by conventional means of control has generally not been economically feasible. Biological control of these pest thistles may thus be the only potentially practical means of control. A number of such studies have been conducted pertaining to this subtribe (Karny, 1963; Harris, 1964; Zwolfer, 1964, 1965a, 1965b, 1967a, 1969a, 1969b; Frick, 1964a, 1966, 1969; Zwolfer and Eichhorn, 1966; Zwolfer and Harris, 1966; Coulson, 1968; Baloch et al., 1971; and others presently in progress).

As a part of this comprehensive investigation for suitable biological control agents to control thistles, a program of study on C. horridus was initiated in Rome, Italy, and Delemont, Switzerland in 1964. This species underwent preliminary testing in Rome

(1964-66, 1968, and 1970) and in Delemont (1964-65). Most of the testing involved starvation tests with the adults. An attempt was made with larval testing in Rome, but adequate techniques for handling of larvae and inoculating them into potential host plants was not developed which precluded a further analysis of the situation. Thus, in this study first instar larval host specificity tests were conducted between September, 1971 and May, 1972, in accordance with one aspect of prerelease studies for screening insects for biocontrol (Harris and Zwolfer, 1968). Specific objectives of this larval testing program were to determine:

1) the host specificity of the first instar among 28 economically and aesthetically important composites and six species of vegetables and two species of forage crops; and

2) the infestation rate for the first instar for all of the above plant species which supported larval development to the second instar in the primary testing program.

In addition, this thesis has incorporated two other aspects for screening insects for biocontrol - a literature survey of the insect's biology and a preliminary study of the mass rearing potential for this species. Biological information from the author's research is presented, while the mass rearing potential was ascertained from an investigation of the methods of adult aestival diapause termination.

LITERATURE REVIEW

The survey of the literature on host specificity is presented as follows: A.) terminology; B) principles; C.) mechanisms; and D.) methods. Section E includes a literature survey of the classification, description, and biology of C. horridus. A description of the geographical distribution and the host plants of the other species in the genus is also presented.

A. Terminology

Food plant range, host plant selection or food plant preferences, and host specificity have all been used synonymously, but there are apparent shades of meaning. Food plant range is the group of plants, large or small, with which an insect is associated (Thorsteinson, 1960). Thorsteinson also pointed out that host plant selection or food plant preference is the resultant effective pattern of plants "chosen" by mechanisms inherent in the species. Host specificity implies a specially designed procedure carried out in the laboratory to determine the potential food plant range. Harris and Zwolfer (1968) concluded that host ranges, selections, and preferences are usually found in nature consisting of fewer plant species than host specificity tests seem to indicate in the laboratory.

Pantophagous, polyphagous, oligophagous, stenophagous, and monophagous refer to the commonly used classifications of phytophagous insects. This series is a gradation relating the

number of kinds of plants eaten, beginning with pantophagy, the omnivorous and probably non-existent grouping to monophagy, almost an equally rare group. Polyphagy is feeding on many plant species from different families; oligophagy is feeding on some species from different families, while stenophagy refers to feeding on a number of species within a family (Jolivet, 1954; Schoonhoven, 1968).

B. Principles

Walsh (1864, 1865, from Dethier, 1954) set forth the olfactory conditioning principle as an opportunity for the development of altered feeding principles in relation to the development of biological races. A "memory" of larval feeding habits predisposes adults of phytophagous species to oviposit on the same species of plant as that upon which they themselves had fed. This idea later developed into the Hopkins Host Selection Principle (Hopkins Host Selection Principle (Hopkins, 1917). As Dethier (1954) stated, given the proper isolating mechanisms (i.e., preferential mating, changing developmental rates in relation to changes in plant distribution, spatial isolation, or others), the chances would be enhanced for the new feeding habits to become germinally fixed.

Fraenkel (1953) stated a theory of host selection as being exclusively by means of 'odd' substances or secondary biochemicals within plants. This idea was pioneered by Verschaeffelt (1910) with his work on Pieris brassicae and P. rapae and their chemical insect-host plant relationships within Cruciferae. Fraenkel (1959) pointed out that the "raison d'etre" of all so-called secondary

biochemicals is solely that of defense against insects and other intruding organisms. Beck (1965) stated that as the oligophagy or monophagy evolved from polyphagy, many insect species were not only able to overcome plant resistance imparted by secondary chemicals, but also evolved tha ability to utilize them as specific secondary clues (token stimuli) to aid in identifying their host plants. This is referred to as active selection.

A third theory presented is that host specificity is determined primarily by the insect's nutritional requirements. This "token stimuli" theory was discounted by insect nutritionists (Lipke and Fraenkel, 1956; Fraenkel, 1953, 1959; House, 1966). However, Beck (1965) cited this as not to be the case since a number of chemicals (token stimuli) have been shown both to be of nutritive value and also to act as feeding stimulants.

House (1966) set forth the principles of insect nutrition into three principles - the rule of sameness, the principle of nutrient proportionality, and the principle of cooperating supplements. The first states that nutritional requirements of insects are much the same irrespective of the systematic position or feeding habit of the species. The second, an amendment to the first, states that metabolically suitable proportions of nutrients are needed for normal nutrition. The third states that supplementary or substitutive sources of nutrients cooperating with the commonly recognized food-stuff of the species are needed to fulfill the nutritional requirements in many insects. House concluded by saying that the best evidence

supports the proposition that food selection and specificity are determined in insects almost entirely by nutritional factors, and that, at least qualitatively, their nutritional requirements are remarkably similar. Finally, House stated that food is not a static factor, but a dynamic one. With regard to the second principle, Atwal and Sethi (1963) found some preference for foodstuffs containing all the essential nutrients in suitable concentrations and ratios for optimum nutrition.

The dual discrimination hypothesis set forth by Kennedy and Booth (1951) and Kennedy (1958) incorporates both "flavor stimuli" or secondary chemicals of specific botanical origin and "nutrient stimuli" or feeding stimulants and deterrents which may or may not be of nutritive value in explaining an insect's response regarding host selection. Beck (1965) pointed out that the hypothesis proposes that host selection comprises both types of stimuli and in so doing has the distinct advantage of flexibility with respect to the changing physiological state of both the insect and the plant. He argued, however, that it is presumptuous to assume that the insect is capable of making nutritional assessments in this regard. As previously pointed out, Atwal and Sethi (1963) have shown that in some cases, insects do show a preference for metabolically suitable proportions of nutrients for optimum nutrition. This lends significance to the dynamic role of host food plants with respect to the array of insects attracted to and thriving upon them.

Painter (1951, 1958) described the antithesis of host selection - plant resistance - as consisting of three categories: "nonpreference" for lack of certain qualities, antibiosis as affecting the biology of the insect adversely, and tolerance, survival under levels of infestation that would severely injure or kill susceptible plants. In terms of evolutionary roles toward host selection, the first probably played a direct role and the second an indirect role (Beck, 1965).

Thorsteinson (1960) introduced a model in light of previous work in the field. The symbolic statement is :

$$F \rightleftharpoons -I\ -D + E_{sn}\ (E_p)$$

where F is the optimal feeding response, -I implies the substrate is devoid of feeding inhibitors, or deterrents -D, and contains the chemical stimulants E, essential to elicit feeding referred to here as E_{sn} (sapid nutrients) and E_p the "piquant" stimulus or catch all.

As a final classification with regard to all principles, Dethier et al. (1960) proposed the following classification of terms with respect to orientation responses directing insect feeding relative to chemosensory responses. An attractant is defined as a stimulus to which the insect responds by orienting movements toward the apparent source. Repellents elicit an oriented response away from the apparent source. Arrestant is a stimulus that causes the insect to cease locomotion in close contact with the apparent

source. Beck (1965) stated that feeding incitant describes a stimulus that evokes the biting or piercing reaction; the converse is a suppressant. Feeding stimulants promote continuous feeding while feeding deterrents prevent continuous feeding or hasten the termination of feeding. These terms are applicable to both physical and chemical stimuli.

Kennedy (1965) reexamined yet another theory that host selection is a catenary process: a chain made up of different responses to different stimuli each of which is received as a result of the insect making the previous response in the chain. As Beck (1965) so aptly stated, the host selection problem is best understood in terms of a concatenation of unit effects.

C. Mechanisms

Mechanisms for host selection can be classified into two types, chemosensory and physicosensory. Schoonhoven (1968) reviewed the chemosensory bases of host plant selection and stated that chemoreceptors are of two types, olfactory and contact (gustation). The first type is located usually on the antennae, but has also been found on the maxillary and labial palpi. The second is generally located on the maxillae, labium, on the tarsi, and the antennae. Gustatory receptors may also be located on the epipharynx, hypopharynx, and labrum. In addition, chemoreceptors have been found on the ovipositor. Much evidence has been accumulated indicating that these receptors sense specific chemicals or plant constituents which may influence feeding behavior. Reception of two

or more chemicals by different receptors can result in mutual inhibition of the response of the receptors involved or in a synergistic interaction. Thus, a dynamic spectrum of sensitivity in terms of a repertoire is not only possessed by the respondent but also by the elicitor; hence, an insect can be tuned to a plant by virtue of its specialized receptor mechanisms. This, coupled with receptors responding to repellents (Ishikawa, 1966) is in accordance with Jermy (1966), that oligophagy in many cases is achieved by avoiding deterrents, reflects the discriminative capacity of the sensory mechanism. Thus, in terms of specificity, Schoonhoven (1968) stated that monophagy and oligophagy could be based on a fairly subtle combination of a number of common plant components, combined with the presence or absence of several secondary substances.

Physical stimuli are also of importance in determining host suitability (Beck, 1965). Evidence indicating specific releasing stimuli for oviposition has been found (Harris, 1964; Zwolfer, 1970a, 1970b; Currie, 1932; Frick and Andres, 1967). The reception of biophysical stimuli usually precludes chemosensory perception and thus may be the initial mechanism for host selection, while the chemosensory stimuli may finely attune the discriminative process for host plant selection.

D. Methods

Methods applied to host specificity tests of a species depend to a large extent upon the insect to be tested and must take into consideration numerous factors, particularly its habitat, life cycle, ethology and ecology. The host range is usually determined by the

stage with the most restricted host range which means that each species must be evaluated individually (Zwolfer and Harris, 1971).

Results can be expressed subjectively or can be objectively quantified. There is a trend toward the latter with subsequent statistical analysis of the data. Sometimes, however, such results are misleading by themselves due to the fallible nature of starvation tests. Huffaker (1962) stated that confinement of an insect to a test plant in a cage does not necessarily provide the safety of the insect for release, and may result in the rejection of a potentially useful species. Harris and Zwolfer (1968) further indicated that 1) confined insects often oviposit on, eat, and survive on more plants than they attack in nature; and 2) insects have evolved specialized mechanisms of orienting to host plants which incorporate many facets of the insect's biology, morphology, and physiology which make its dependence on its host obligatory, but this dependence does not necessarily carry over to laboratory conditions. For these reasons, Harris and Zwolfer set forth the following guidelines for prerelease studies on insects proposed for biocontrol of weeds: 1)a study of the insect's biology with particular attention to adaptations likely to restrict its host range; 2)a review of the plants attacked by related insects; 3)a determination of the laboratory host range of the insect; 4) an investigation of the chemical and physical basis of host recognition; and 5) starvation tests on economic plants to confirm the limits of the laboratory host range. In addition, they proposed that plants tested include those related

to known hosts, host plants of related insects, those plants for which occasional records have been obtained, and those plants which have characteristics in common with the host which may make them acceptable. If information regarding host plants is lacking, a greater range of plant taxa should be tested.

The methods applied in terms of insect feeding on economic host plants, in biocontrol screening tests, and insect sensory mechanism research have yielded two methodological designs; one encompassing the plant in toto and the other involving bioassay of plant extracts. The former method has been developed into a diversified array of techniques, each relatively specific to the experimental conditions relevant to the life stage of the insect being studied.

Zwolfer and Harris (1971) presented an excellent review of many techniques which have been used in biocontrol screening tests. Other techniques used with insect feeding upon economic hosts have been added to broaden the perspective in this area.

Primary methods for evaluating adult leaf eating, oviposition as an indicator of host selection, and larval feeding are presented in Tables I - III respectively.

Bioassay of feeding extracts involves to a large extent insect behavior in relationship to a presented extract stimulus. The field has been reviewed by Thorsteinson (1955; 1960), Hsiao (1969), Jacobson (1966), and Schoonhoven (1968) among others. Primary methods for the bioassay of feeding extracts are presented in Table IV

TABLE I. Primary methods of evaluating adult leaf feeding.

METHOD	AUTHOR
Area consumed	Harris, 1964 Hawkes, 1966 Zwolfer and Eichhorn, 1966 Frick, 1971
Area consumed in terms of feeding units	Zwolfer, 1965a Hawkes, 1966 Zwolfer and Eichhorn, 1966 Zwolfer, 1967a Zwolfer, 1968 Zwolfer, 1969a Frick, 1970a Frick, 1971
Area consumed measured photometrically	Kogan and Goeden, 1969
Detection of feeding and quantification by means of radioisotopes	Maddox and Resnick, 1969
Counting or weighing fecal pellets as an indication of feeding	Harris, 1963
Longevity upon the plant as an indicator of suitability as a host	Zwolfer, 1965c Zwolfer, 1967a Zwolfer, 1969a Dunn, 1970a, b, c Frick, 1970a Frick, 1971
Proportion of individuals surviving in a given period	Harris, 1964
Subjective analysis of feeding	Andres and Angalet, 1963 Force, 1966a, b Jermy, 1966 Zwolfer, 1967a

TABLE II. Primary methods of evaluating oviposition as an indicator of host selection

METHOD	AUTHOR
Oocyte formation or oogenesis	Andres and Angalet, 1963 Force, 1966a Baloch et al., 1967
Egg production	Zwolfer, 1969b
Number of eggs oviposited in preference tests	Harris, 1963 Zwolfer, 1970a Boling and Pitre, 1971
Number of eggs oviposited in single plant tests	Force, 1966a,b Hawkes, 1966 Frick and Andres, 1967 Zwolfer, 1967a Kirk et al., 1968 Kok, 1968 Dunn, 1970a, b, c Frick, 1970a Zwolfer, 1970a Kamburov, 1971
Subjective measure of host acceptance for oviposition	Parker, 1964 Zwolfer and Harris, 1966 Zwolfer, 1967a Zwolfer, 1970a
Duration of oviposition	Hawkes, 1966 Frick, 1971
Number of oviposition sites in a preference test	Harley and Kunimoto, 1969
Duration of probing on host plants	Zwolfer, 1970a
Oviposition response in terms of subjective probing	Zwolfer, 1970a

TABLE II (Continued). Primary methods of evaluating oviposition as an indicator of host selection.

METHOD	AUTHOR
Probing time on offered shape and color stimuli	Frick, 1970b
Oviposition site selection	Zwolfer, 1967a Zwolfer, 1970a
Viability of eggs	Frick, 1970a

TABLE III. Primary methods of evaluating larval feeding.

METHOD	AUTHOR
Measuring length of mines	Schroder, 1969
Weight of food consumed	Waldbauer, 1962
Subjective measure of larval feeding	de Wilde, 1958 Oatman, 1959 Gupta and Thorsteinson, 1960
Larval weight gain per time period	Beck, 1956a Davis, 1957, 1958 Waldbauer, 1962
Number of feces per larvae	Waldbauer and Fraenkel, 1961
Larval head capsule width measurements and nutritive effect	Branson and Ortman, 1967a, 1970
Larval head capsule width measurements and prepared diets with varying concentrations of nutrients	Beck, 1956a Beck, 1956b
Larval head capsule width measurements and inhibition of larval growth on plant varieties	Beck and Smissman, 1960
Larval head capsule width measurements and diets containing varying concentrations of plant material	Beck and Stauffer, 1957
Subjective larval growth	Waldbauer and Fraenkel, 1961
Larval mortality or vitality per time period	Beck, 1956a Waldbauer and Fraenkel, 1961

TABLE III (Continued). Primary methods of evaluating larval feeding.

METHOD	AUTHOR
Larval mortality with exposure to plant substrate	Branson et al., 1969
Percent dead in a given instar	Swailes, 1960
Survival or percent survival	Davis, 1957, 1958 Kok, 1968
Percent not established on food	Swailes, 1960
Percent established on food	Beck, 1956b
Development of larvae on food substances	Davis, 1957, 1958 Parker, 1960 Harris, 1963, 1964 Zwolfer and Harris, 1966 Zwolfer, 1967a Baloch et al., 1969 Frick, 1970a Frick, 1971 Kamburov, 1971 Zwolfer, 1972
Percent pupated	Swailes, 1960 Kok, 1968
Weight of puparia and number of days to pupation	Swailes, 1960 Waldbauer, 1962
Percent adult emergence	Kok, 1968 Branson and Ortman, 1970
Adult weight after emergence	Branson and Ortman, 1967a
Head capsule width of adults after emergence	Branson and Ortman, 1967a Branson and Ortman, 1970
Number of eggs oviposited and their viability	Waldbauer, 1962 Branson and Ortman, 1967a Branson and Ortman, 1970 Frick, 1970a

TABLE IV. Primary methods for bioassay of feeding abstracts.

METHOD	AUTHOR
Smearing extracts on test material	Grevillius, 1905 Verschaffelt, 1910 Dethier, 1941
Screen tests	Dethier, 1937
Tube tests (olfactometer)	McIndoo, 1935 Munakata et al., 1959
Leaf disc test or sandwich test	Gupta and Thorsteinson, 1960 Jermy, 1961 Jermy, 1964, 1965
Vacuum leaf infiltration tests	Krotkov, 1947 Kuhn and Gauhe, 1947 Harris and Mohyuddin, 1965 Simons et al., 1968
Use of elder pith as a mechanical support for extracts	Raucourt and Trouvelot, 1936 Chauvin, 1945 Harris, 1963 Harris and Mohyuddin, 1965
Use of plain filter paper	Dethier, 1941 Thorpe et al., 1947 Chauvin, 1951
Use of charred filter paper	Yamamoto and Fraenkel, 1960 Thorsteinson and Nayar, 1963 Niimura and Ito, 1964 Harris and Mohyuddin, 1965
Use of agar diet substrate	Beck, 1956a,b, 1957, 1960 Beck and Stauffer, 1957 Beck and Smissman, 1960 Vanderzant et al., 1962 Nayar and Fraenkel, 1963 Harris and Mohyuddin, 1965
Use of artificial diet plus leaf powders or extracts	Thorsteinson, 1953 Jermy, 1958, 1961 Schoonhoven, 1967 Hsiao and Fraenkel, 1968c Hsiao, 1969

E. Biology

Appendix A presents a bibliography for C. horridus which includes a major portion of the literature for this species.

A genus and species description for the adults is given by Hoffman (1954). Plate I is an adult shown clasping to a Carduus nutans leaf tip. Plate II shows the ventral aspect of an adult female and illustrates the relative size of the third instar and the first instar to the lower right of the third instar. The scale of measure is in millimeters.

Geographical Populations

Information on the biology of C. horridus has been recorded both from Central Europe (Everts, 1903; Hustache, 1923; Hoffman, 1954; Auber, 1960; and Sherf, 1964) and in Southern Europe (Italy) (Frick, 1964a, 1966, 1969). With respect to life history data from Central Europe, Hustache (1923) and Auber (1960) reported finding the adults as early as April, and then again in August and September. Hoffman (1954) reported that pupation occurs in O. acanthium in July and transformation occurs in August and September with the adults hibernating through the winter. Sherf (1964) indicated that the larvae were found in a single galleries in the crown of O. acanthium in June, pupation occurred in July and August in pupation chambers in the soil with adults emerging in September. There is one generation annually.

A synopsis of these citations indicates that overwintering adults apparently emerge as early as April from their hibernal sites.

Plate I. Adult C. horridus clasping to a C. nutans leaf tip.

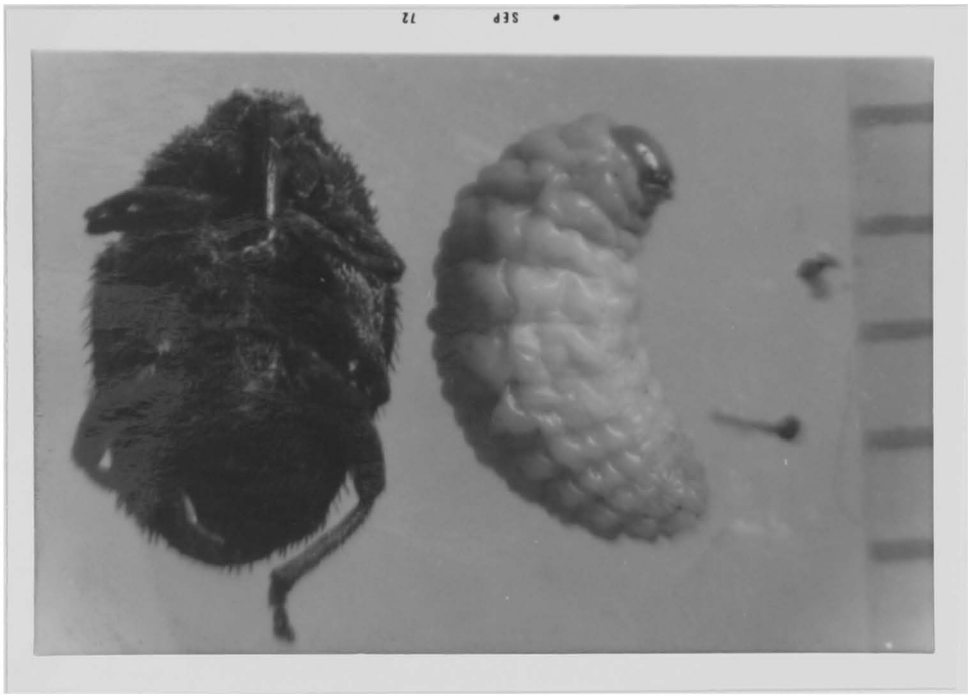

Plate II. Ventral aspect of an adult C. horridus female and lateral view of a third instar and a first instar.

Emergence probably continues through the middle of May with the adults feeding and mating throughout this period. Oviposition apparently takes place from the middle of May through June with the larvae pupating in July and August and emerging as adults in September. Teneral adults probably feed for a period prior to hibernation to increase food reserves. This seasonal life cycle suggests that the Central European populations of C. horridus probably undergo an obligatory hibernal diapause with one generation annually.

Frick (1966, 1969) provided life history information for C. horridus populations in Southern Europe (Rome, Italy). He indicated that larvae were found in the crowns of thistle plants in the field from February through June with adults emerging from March through July. Larvae were found in C. pycnocephalus, C. lanceolatum, Galactites tomentosa, and in C. nutans 12 to 18 larvae per crown were not uncommon with a maximum of 33 in a large crown. Teneral adults were observed only from April through June and were primarily found on Galactites tomentosa, C. nutans, and C. pycnocephalus. In the latter part of June they were much less frequently seen in the field apparently due to an aestival diapausing behavior which is an adaptation to the dry summer type of Mediterranean climate. Adults reappeared from their aestivation sites in the fall and early winter, but it was thought that adults may be active prior to this time by remaining inconspicuously around the bases of thistle rosettes near the soil. Oviposition was observed to begin in mid to late December and continued to early March. This seasonal life cycle suggests that the Southern

European populations of C. horridus probably undergo either an obligatory or facultative aestival diapause with the information stated by Frick as to the scarcity of weevils in the field during the summer suggesting that perhaps the former may be the case.

The life histories for each of the geographical populations mentioned lends significance to the belief that C. horridus is comprised of at least two geographical populations, a northern and a southern population which by virtue of their latitudinal and climatic differences are both geographically and seasonally isolated from each other.

Since latitude primarily determines the photoperiod of a given location, the greater the difference in latitude, the greater will be the differences between the seasonal climates of two distant locations. Conversely, the closer the two locations, usually the more similar are the climates. Thus, throughout the entire geographical range of C. horridus clusters comprised of gradations of adapted geographical populations suited to a particular locale should be found. At each extreme in the geographical range, populations should exhibit contrasting adaptations to the local environment. This may be the case with the two populations mentioned above, in which, by virtue of their climatic differences, have adapted two distinctly different life cycles. Thus, the exposure to two different photoperiod regimes as well as the climatic factors of each locale (Central or Southern Europe) has resulted in differences in phenology and subsequently may alter the host plant specificity with

regard to available potential hosts. This difference is indicated in the host plant range given below. It should be added that these ecological adaptations which include adaptation to day length and temperature in the environment are manifested as phenological adaptations which function to synchronize the biology of the insect with that of its host plants.

It cannot presently be stated that these two geographical populations are distinct races or subspecies because the necessary requisites by definition have not as yet been tested. However, I suggest that the status of race may be found to be fitting, since there is little doubt as to the existence of differences in their life histories. The question remains as to whether these differences are great enough to prevent sympatric populations from successfully overlapping and subsequently interbreeding. Mayr (1963) stated that in cases of speciation by seasonal isolation where a species has developed two different life cycles, this is accomplished primarily by geographical isolation and secondarily by seasonal differences in the reproductive cycle. One might then conclude that the duration of separation of the two populations will determine the degree of speciation acquired to date.

Mayr (1963) pointed out that it is no coincidence that all the cases of presumed sympatric speciation by temporal isolation concern genera rich in sibling species. In the genus Ceuthorrhynchidius, C. horridus and C. urens are considered to be sibling species (Zwolfer and Harris, 1966). These two species are more than likely exemplary

of the successful speciation by geographical and seasonal isolation since the former is distributed primarily in Southern and Central Europe while the latter is distributed in the Caucasus - Mediterranean region. The two species are again sympatric in the Mediterranean, but the acquisition of isolating mechanisms by each prevents hybridization despite the many morphological similarities between the two species. Under similar circumstances thousands of years from now, the two geographical populations of C. horridus may acquire a similar set of isolating mechanisms precluding each to the taxonomic rank of species.

Geographical Distribution of C. horridus

The geographical distribution of C. horridus is mostly European - Atlantic - Western and Northern Mediterranean since it occurs over all of France, southern Britain, Netherlands (not indigenous), in Westphalia (Everts, 1903), Germany, Switzerland, Austria, Hungary, Transsylvania (Romania) where it is not infrequent (Seidlitz, 1891), and all of Italy, Corsica, Sardinia, and Sicily (Luigioni, 1929). C. horridus is found in the plains and mountains of France and has been reported from Isere and from the Salette Mountains at an altitude of 1800 meters on August 15, 1918 (Hustache, 1923). It is usually located on its host plants which occur in barren, waste lands (Auber, 1960).

Host Plants of C. horridus

C. horridus larvae have been found in Central Europe in the stem bases of Onopordum acanthium (Hoffman, 1954; Sherf, 1964), and

in Carduus acanthoides (Zwolfer, 1965). In Southern Europe they have been found in the crowns of Carduus nutans, C. pycnocephalus, Cirsium lanceolatum, and Galactites tomentosa.

The adults were found in France on Carduus crispus, C. nutans, and Cirsium arvense (Hoffman, 1954). In Delemont, Switzerland, adults have been taken from Carduus acanthoides, C. crispus, and C. nutans. Frick (1966, 1969) reported collecting the adults in Italy off C. nutans, C. pycnocephalus, and Galactites tomentosa.

C. horridus has also been reported from chardon (thistles) (Perris, 1877), auf Disteln (on thistles) (Stierlin, 1894), on thistles (Joy, 1933), sur les Carduacees (on the Carduinae) (Portevin, 1935), and on Onopordum (Sherf, 1964); Onopordum, Carduus, Cirsium, etc. (Fowler, 1891; Everts, 1903; Porta, 1932). More specifically it has been reported on Onopordum acanthium, Carduus nutans, Cirsium arvense (Hustache, 1923; Hoffman, 1954). In addition Hustache reported it from Carduus crispus. Linssen (1959) reported C. horridus as occurring on Plantago (Plantains); Schaufuss (1916) reports it to occur on Carduus, Cirsium, and Carthamus; and Sherf (1964) indicates that Ceuthorrhynchidius sp. larvae inhabit plant species of the family Plantaginaceae, Compositae, and possibly also Cruciferae. Unfortunately some of the reported hosts are questionable, others the result of some taxonomists' whim to include or exclude certain species in the genus which reached a peak of 24spp. (Heyden, et al., 1883, 1891), which is all misleading and time consuming to the biocontrol researcher.

Description of the Geographical Distribution and the Host Plants of the Other Species in the Genus Ceuthorrhynchidius Jacq. du Val.

Dalla Torre and Hustache (1930) reported 17 species in the palearctic genus. These are presented in Table V along with their distribution and reported hosts (Table VI) as determined from some of the literature citations.

Species in the genus are palearctic ranging from Britain through Europe to Sweden, Finland and Russia, south to North Africa and west through eastern Europe, Greece, Turkey to the Caucasus Mountains with C. hypocritus occurring in Japan. Plants attacked include the Carduinae (Compositae) by C. horridus and C. urens; Plantago (Plantagaceae) by C. baldensis, C. Barnevillei, C. Dausoni, C. hassicus, C. Thalhammeri, C. troglodytes, and C. rufulus; Anthemis (Compositae) by C. hassicus and C. hystrix; Achillea (Compositae) by C. Barnevillei and C. hassicus; Artemesia (Compositae) by C. rufulus; Reseda (Resedaceae) by C. rufulus; and Picnomon acarna Cass. (Compositae) by C. urens in Algeria. Seven other species are lacking host descriptions. The genus thus appears rather oligophagous in food habit encompassing only three plant families, with species which are quite stenophagous and some appear to be monophagous. These are encouraging qualities as potential biocontrol agents for thistles and possibly even for plantains.

TABLE V. Geographical Distribution of Species of the Genus Ceuthorrhynchidius.

Ceuthorrhynchidius Jacq. du Val

C. baldensis - Schultze, 1896; Mt. Baldo, Grand Snt. Bernard, Bosnie, Alps Fr., Alps Pennine, Hongrie, Herzegovine, Styrie

C. Barnevillei - Gren, 1866; Hautes Pyrenees, Hautes Alps, Baviere, Autriche, occ. Hungary, Croatie, Tic (Chiasso), Pyrenees - Orientales, Basses - Alps, Jura, Germany, Transcaucasie, Caucase, Denmark, Suede, Angleterre, Alp. coz (Monginevro).

C. Bedeli - Schultze, 1897; Algerie.

C. bellus - Reitt, 1890; Araxes

C. campanellae - Schultze, 1895; Croatie, Bosnie, Dalmatie, Caucase.

C. centrimacula - Schultze, 1899; Algerie, Grece, Sicile, Syrie.

C. Dawsoni - Ch., 1869; Angleterre, France, Espagne, Scotland, Ireland, littoral maritime, Morocco, Chaouia (de Bordes).

C. hassicus - Schultze, 1903; Allemagne, Hongrie, Bosnie, Poland, rare in France.

C. horridus - Panz, 1801; Europe centrale et meridional, Transslvanyia, Italie, Sicile, Sardinia, Corse, Angleterre mer.

C. hypocritus - Hust., 1916; Japan.

C. hystrix - Perris, 1852; France mer and occ Italie, Grece, Espagne, Algerie, Sardinia, Morocco.

C. magnicollis - Schultze, 1903; Algerie, Tunisie, Angleterre, France, Allemagne mer, Autriche, Hongrie, Italie, Turquie, Caucase, Algerie.

C. Spurnyi - Schultze, 1901; Tyrol, Vallarsa: Triolis m.

C. Thalhammer -Schultze, 1906; Alps occ., Hongrie, France mer, Loire - Inferieure, Bouches - du - Rhone, Corsica.

TABLE V (Continued). Geographical Distribution of Species of the Genus Ceuthorrhynchidius

C. troglodytes - F., 1787; Europe, Maroc, Algerie, Siberie, Britain, Italy, I. Lussino, I. Elba, Corsica, Malta, Europe to Sweden and Finland.

C. urens - Gyll., 1837; Europe mer, France mer, Espagne, Portugal, Italie, Caucase, Syrie, littoral Mediterranean, Sicily, Tosc, Mar., Puglae, Alpes - Maritimes: Theoule, Pyrenees - Orientales.

C. rufulus - Dufour, 1851; Netherlands, Belgium, I. Elba, I. Capri, Puglie, Sicily, Britain, Italy, Germany m. Austria, Hungary, Turkey, Caucase, Algeria, Pyrenees - Orientales, Jura, Algeria, Angleterre.

TABLE VI. Recorded host plants of some Ceuthorrhynchidius species.

Ceuthorrhynchidius Jacq. du Val

C. baldensis: on Plantains

C. Barnevillei: Plantago alpina, Achillea millefolium

C. Bedeli: hosts not known

C. bellus: hosts not known

C. campanellae: hosts not known

C. centrimacula: hosts not known

C. Dawsoni: Plantago maritima, P. coronopus, P. arenaria

C. hassicus: Plantago lanceolata, Anthemis mixta, Achillea millefolium, P. major

C. horridus: Carduus nutans, C. acanthoides, C. crispus, C. pycnocephalus, Cirsium arvense, Onopordum acanthium, Galactitis sp., Carthamus sp.

C. hypocritus: hosts not known

C. hystrix: Anthemis mixta

C. magnicollis: hosts not known

C. Spurnyi: hosts not known

C. Thalhammeri: Plantago maritima, P. coronopus

C. troglodytes: Plantago lanceolata L.

C. urens - Cirsium arvense, Picnomon acarna

C. rufulus - Plantago lanceolata, P. alpina, P. maritima, P. lagopus, Artemesia maritima, Reseda sp.

MATERIALS AND METHODS

Ceuthorrhynchidius horridus adults involved with this investigation were collected as diapausing teneral adults from May 12 to May 16 in Rome, Italy by Mr. Paul Dunn of the USDA, ARS Entomology laboratory.

On May 26, 1971 the Blacksburg Quarantine Laboratory received 416 males, 355 females and 33 dead, non-sexed individuals for a total of 804 weevils. The adults were immediately set up in groups of 50 (unsexed) in 380 cc plastic cages with a 100-mesh screen top and supplied with one Carduus nutans leaf per cage. The leaves were cut from greenhouse grown thistle rosettes, their basal portions removed leaving a protruding section of midrib 3 cm long. The apical portion of the midrib nearest the cut leaf edge was wrapped securely with approximately 1.5 cm widths of absorbent cotton and securely inserted into a 1.35 cm hole bored into a #10 wax-covered cork. A 38 ml vial filled with distilled water was inserted from beneath into the 1.35 cm hole providing the cut leaf with a water supply. The 16 prepared cages were then placed upon a wooden platform and initially placed in a 70° constant temperature cabinet under a 16 hour photoperiod provided by two twenty inch 15 watt cool white flourescent tubes. The light intensity, determined by a Weston Model 756 photometer, measured 30 and 100 footcandles inside the base and at the outside top of the cages respectively. The relative humidity was between 40 and 50 percent.

From May 26, 1971 to June 17 cages were checked periodically and supplied with fresh leaf material as needed. A record was kept of the date, number, and sex of all weevil mortality occurring during the period with respect to each cage. On June 17, 739 living weevils were sexed and separated into four groups of approximately 100 males and 85 females per group. Each group was then further divided into four replicates averaging 25 males and 21 females. These groups were used in an experiment to determine the most efficient means of breaking diapause. Each group was housed under a different set of photoperiod and temperature conditions. This experiment is described in Part II.

Throughout the duration of this investigation, all adults with the exception of those involved in two independent adult feeding experiments were provided with both greenhouse and field grown leaves of Carduus nutans. At each feeding, information regarding the date and number and sex of all dead weevils was recorded for each cage.

In July, 1971 all 380 cc cages were replaced with clear plastic one-quart cages. A 100 mesh screen covering a 5 cm diameter hole in the top and two 4 cm diameter holes on opposite sides plus a 1.35 cm hole midway between the cage top and bottom made them suitable cages. A 4.5 cm hole was cut into the translucent plastic lid to fit a #10 wax-covered cork, which had been bored out to securely hold a 38 ml vial, 2.5 cm in diameter by 9.5 cm long. These changes were necessary to overcome a condensation problem, to provide adults with additional feeding material, and to lengthen the necessary time

period between fresh provisions of plant material. Light intensity was halved to 690 footcandles and the thermoperiod reduced from 80° - 60° F to a 70° - 50° F schedule for cages housed in the Model E-7-H Environmental Chamber. This was done to correspond to the prescribed photoperiod - thermoperiod regime used for the diapause termination experiment. All adults were housed in this chamber by September 16, 1971 except for one-half of one treatment which was placed in the chamber on October 10, 1971.

Cages, lids, corks, and vials were cleaned periodically to maintain a clean system. Stem rots of cut leaves were eventually eliminated by autoclaving all vials and cleansing corks with each fresh provision of plant material. All plant material removed from cages (i.e., leaves, cotton, etc.) was autoclaved to uphold quarantine regulations.

A. Host Specificity

Care and Handling of Eggs

The first group of C. horridus adults broke diapause September 5, 1971. Eggs were dissected from the midrib of the thistle leaves and placed on non-sterile absorbent cotton in 38 ml plastic cups by means of micro-dissecting forceps. Transfer of eggs on the tip of the forceps was facilitated by moistening the tips with distilled water. One piece of cotton was tightly packed within the cup to prevent penetration by hatching larvae. The cotton was saturated with distilled water and then compressed lightly while tipped to allow excess water to flow out. Eggs were placed upon the cotton surface in a uniform,

orderly manner to facilitate counting. The egg rearing cup was closed with a tightly fitting lid which was provided with a 1.2 cm diameter opening covered with 100 mesh screen. The opening allowed some evaporation and air flow. The cups were labelled with the following information: treatment and replicate number of the source adults, date, and number of eggs in the cup. The eggs were subjected to a 10 hour photoperiod, the rearing room was maintained at a constant temperature of 70 $\pm$ 3° F, 40-50% relative humidity, and approximately 500 footcandles of light intensity (as determined by a Weston Model 756 photometer). Light was supplied by a bank of four General Electric 96 inch PG 17 cool white power groove flourescent lights.

Care and Handling of Larvae

Upon hatching, the first instars were usually found on the underside of the lid. Lids were carefully removed and the larvae transferred to a white cardboard lid for observation. The best procedure was to allow each young larva to crawl up on a point of the forceps while being viewed under a 10X binocular dissecting microscope. The larva was allowed to crawl off the point onto the observation cap while still under the scope. This procedure was laborious but highly efficient.

On the observation cap the larval head capsule width and body length was determined using an ocular micrometer. Body length was defined to be the length from end to end at the maximum stretch of the gait. All measurements were determined using the same microscope and eyepiece throughout. Records of body length and head capsule width

were kept. In addition number and date of hatch was recorded enabling percent of non-viable eggs to be determined as well as the probable cause.

Inoculation Technique

The criteria for selecting healthy larvae for inoculation was as follows: 1) only 1-2 day old first instars were used; 2) of these, only healthy larvae able to crawl in an apparent normal fashion were selected; and 3) all deformed larvae were excluded from testing.

Using the technique mentioned above, larvae were transferred and allowed to crawl head first into 1.5 cm cut sections of plain hematocrit capillary tubing with an internal diameter of 1.1 to 1.2 mm. Plant cores 3-5 mm long were bored from leaf midribs and plant stems of test plants using appropriately cut 1.4 mm diameter by 15 cm long Pasteur disposable pipets. A second pipet with a smaller bore was employed to suck excess plant juices from the hole. The capillary tube containing the larva was placed in the hole with the anterior end of the larva toward the plant. Warm melted Gulf parafin wax from a double boiler water bath was applied with a microspatula to the open end of the tube, to the tube-plant junction, and to the underside of the midrib in the proximity of the bore. Care was exercised to prevent coring through the midrib or stem; waxing of the underside of the stem was done to help prevent larvae from continuing to bore through the cuticular wall of the plant and escaping. These precautions were intended to inhibit escape, and allowed observation

of escapes when such an event occurred. Records were kept on date of inoculation, date checked, larval measurements, replicate number, and plant species inoculated. Inoculation of test species was carried out as the plants and larvae became available; thus selection of a test plant on a particular day did not follow any type of designed selection procedure.

Post Inoculation Technique

At regular 5-day intervals from the day of inoculation, plant midribs and stems were dissected, exercising extreme care not to crush the larva or destroy the larval mine. Once found, the larva was transferred and measured as above, its tunnel length measured under the 10X binocular scope using the ocular micrometer, and its condition recorded. Death was recorded as well as probable cause (fungi, rot, etc.) and the larva was preserved in 95% ethyl alcohol. A record of larval escape via exit mines was kept. These larvae were not replaced unless cause of escape was determined to be due to a poor initial inoculation technique. Plants in which larvae had emerged from the leaf or petiole were set aside for observation of possible reinfestation at another site. Examples of reinfestation were noted and verified by subsequent dissection. All larvae which were accidentally crushed were replaced and the data applying to the initial inoculation was excluded. When found alive, the vitality of the larva was noted (active or passive) and the larva was reinoculated (employing the same technique) into a fresh leaf midrib or plant stem of the same plant. It was intended that

different leaves of the same plant would be used for each replicate to determine both experimental error and sampling error, but this could not be done due to a shortage of plant material.

As the experiment progressed, larval instars were noted and changes in the diameter of the inoculation tube made to correspond to the increasing size of the larva.

All inoculated plants were placed upon laboratory tables approximately 81 cm from banks of G.E. cool white power groove flourescent lights. The light intensity 15-25 cm above the table measured 400-500 footcandles and 66 cm above the table measured approximately 1500 footcandles. All plants were exposed to this range of light intensity. The temperature was a constant 70° $\pm$ 3° F and 40-50% relative humidity. Plants were exposed to at least a 10 hour photoperiod, but this was not kept consistently since the nature of the work required time beyond the 10 hour limit and the facilities could not accommodate an alternate setup. Subsequently, photoperiods varied from 10 to 18 hours with the shorter photoperiod of 10 or 12 hours being more consistently adhered to. During daily watering of the plants, care was exercised to avoid overwatering and to avoid wetting the inoculation sites.

Experimental Design of Test Plants

The experimental design was a completely randomized design replicated three times.

The plants were divided into groups as follows:

Group I - Cynareae

1. Carduus nutans - musk thistle.
2. C. acanthoides - curl thistle.
3. C. pycnocephalus - Italian thistle.
4. C. horridissimus.
5. Silybum marianum - milk thistle.
6. Cirsium arvense - Canada thistle.
7. C. vulgare - bull thistle.
8. Carlina acaulis.
9. Onopordum acanthium - scotch thistle.
10. Xeranthemum annum.
11. Centaurea montana - montana thistle.
12. C. nigrescens - star thistle.
13. C. cyanus - cornflower.

Group II - Economic composites

1. Carduus nutans - musk thistle.
2. Cynara scolymus - artichoke.
3. C. cardunculus - cardoon.
4. Helianthus annus - sunflower.
5. Lactuca sativa - head lettuce.
6. Centaurea sultana - dusty miller.

Group III - Other composites

1. Carduus nutans - musk thistle.
2. Chrysanthemum sp. - chrysanthemum.
3. Senecio arenarius.

4. Aster sp. - aster.
5. Inula sp.
6. Cichorium intybus - chicory.
7. Cichorium endiva - endive.
8. Tagetes erecta - African marigold.
9. Artemesia vulgaris - mugwort.
10. Helichrysum sp. - strawflower.
11. Taraxacum officinale - dandelion.

Group IV - Economic plants

1. Carduus nutans - musk thistle (Compositae).
2. Capsicum frutescens - pepper (Solanaceae).
3. Lycopersicon esculentum - tomato (Solanaceae).
4. Raphanus sativus - radish (Cruciferae).
5. Beta vulgaris - beet (Polygonaceae).
6. Daucus carota Var. sativa - carrot (Umbelliferae).
7. Brassica oleracea botrytis - cauliflower (Cruciferae).
8. Zea mays - corn (Gramineae).
9. Medicago sativa - alfalfa (Leguminosae).

All plants were grown from seed in the greenhouse with the exception of some species transplanted from the field to the greenhouse when seed was lacking.

Second Larval Transfer Test

In this test, plant species which supported larval development to a second instar from the primary host specificity experiment were used as test plants. This would include the following: Lactuca sativa, Carduus acanthoides, Cirsium vulgare, Cirsium arvense, Onopordum acanthium, Xeranthemum annum, Centaurea nigrescens, and Carduus nutans. Due to a shortage of available test plant species, Xeranthemum annum, Centaurea nigrescens, Cirsium arvense, and Lactuca sativa were excluded from the test. Cynara scolymus and Helianthus annus were included as test plants since they are important economic species while Carthamus tinctorius was not available for testing. In addition, Carduus horridissimus was included since it was available and is an important southern thistle. Thus, those species tested included: Carduus nutans, C. horridissimus, C. acanthoides, Cirsium vulgare, Cynara scolymus, Onopordum acanthium, and Helianthus annus. Each species was inoculated with 15 first instars by the procedure mentioned previously. At the end of 30 days, all plants were dissected and the number of larvae and instars infesting the plant determined as well as the vitality of the plant. All larvae found were preserved in 95% ethyl alcohol.

Host Specificity Index and Index of Survivability

Because of the inconsistencies in certain test result criteria, I chose to incorporate several variables. The technique of incorporation was adapted in part from Zwolfer and Eichhorn (1966). Two new indices are defined for the first time: the larval host

specificity index (HSI) and the larval index of survivability (IS). The equation for calculating the host specificity index consists of two parameters as follows:

$$HSI = (FU)\ (L)$$

where FU is the feeding unit defined as the mean tunnel length (mm) mined per larva as depicted in Table IX for each of the plant species tested. The feeding unit is calculated by summing the tunnel lengths mined for all three replicates and dividing by 15, the total number of larvae inoculated in all three replicates. "L" is the longevity, defined as the mean number of days to the death of the last larva. It is calculated for each species by summing longevity figures for all three replicates for each plant species tested from Table IX and dividing by three. The index is calculated by determining the product of FU and "L" which could be defined as the feeding intensity (FI). Next, the mean feeding intensity for the four Carduus nutans treatments is determined by summing the four figures and dividing by four. With the standard calculated, C. nutans is defined to be 100 percent on the host specificity index scale and the HSI percent for the other plant species is determined with respect to the standard. The last step is to rank the plant species in descending order from highest to lowest and place them in the respective logarithmic categories: (a) most preferred species ($HSI > 100\%$), (b) High (HSI 30-100%), (c) Moderate (HSI 10-29.99%), (d) Low (HSI 3-9.99%), and (e) very low ($HSI < 3\%$). The HSI is used only in relationship to the entire experiment referred to as total.

Table XII gives the HSI rankings along with the number of larvae which were able to develop to the second and third instar. These two parameters are included to point out the extent of development of the larvae among the species tested and are therefore not a part of the equation, but add greater meaning to the rankings.

The index of survivability (Table X) is analogous to the previous index and might also be termed the first instar relative host specificity index, but by definition it is different since it spans only the first five days of the total test period. It will subsequently be referred to as the index of survivability. It is defined by the equation:

$$IS = (\%A)\ (\%BLG+)$$

where %A is the percent of larvae alive after the first five day exposure to the test plant, %BLG+ is the percent of larvae showing a positive body length growth for the same five day exposure period to the test plant. The product of these two parameters yields the first five day probable survivability percentage. The standard is calculated as above for the HSI and ranked in a similar fashion.

Table XIII gives the IS rankings along with the feeding units (FU) which is the mean tunnel length mined per larva for the same five day exposure period. It is calculated in a similar fashion as mentioned above for the HSI. This parameter although not a part of the equation for determining IS is included mainly as a means of comparison to demonstrate the relative amount of feeding among the plant species tested during the first five day exposure period.

Once the indices are calculated and the plant species are ranked into the five logarithmic categories based on the standard: Carduus nutans (HSI = 100%; IS = 100%), the degree of association between the two indices is desired. To determine this, a correlation coefficient was calculated along with the t-test for the null hypothesis p=0. From Table XI, r=.9541 with a highly significant t value of 26.00, thus rejecting the null hypothesis p=0. This high degree of association between the two indices is indicative of the trend that those larvae which survived and demonstrated growth by virtue of increasing body length for the first five days on a test plant are ranked with a corresponding consistency with those larvae which are categorically ranked according to the amount of tunnel length mined and their longevity upon the same test plant for the entire test period.

B. Larval Measurements

Head capsule and body length measurements were made as mentioned in the care and handling or larvae section of the materials and methods. Measurements were made for all first, second, and third instars which resulted from the testing programs and a mean, confidence limit, and range determined.

Second Transfer Test

Larval measurements were made of all larvae resulting from this test at the end of the 30 day inoculation period. The number of larvae measured, the mean and confidence limits were determined for each species of plant tested.

Primary Host Specificity Test

Larval measurements were made of all larvae able to develop to the second and third instar on the plant species tested. The number of measurements, the mean head capsule width, the body length after molting to each instar, the mean number of days to develop to each instar, and the mean tunnel length mined by larvae up to each instar were recorded.

C. Oviposition Observations

Observations were made on the oviposition behavior of C. horridus as to where eggs were oviposited on a C. nutans leaf, the usual number found, and the appearance of the oviposition site.

A starvation and oviposition test with five males and five females per test plant was begun on November 8, 1971. Test plants consisted of Carduus nutans, C. acanthoides, Cirsium vulgare, Helianthus annus, Carthamus tinctorius, Lactuca sativa, and Cynara scolymus. The test was replicated once. Test plant leaves and adults were housed in plastic cages mentioned in the introductory section of the materials and methods and procedures of feeding were similar to the procedure outlined in that section except fresh leaves of seven different species were given to respective treatments every three days.

Egg hatch time from day of oviposition was determined by recording the day eggs were oviposited, the number of eggs hatched per day and determining the span of time required for eclosion for 435 eggs. By recording the number of eggs hatched from a total number

oviposited and recording the various causes preventing eggs from eclosion, precent hatch for 2,346 eggs could be determined as well as the numbers succumbing to various causes.

RESULTS

An analysis of variance on the lengths of tunnels mined by larvae (Table VII) reveals that plant species within groups I and III are significantly different at the .05 level for the first five day period of the test while plant species within groups II and IV are significantly different at the .01 level. Treatment means based on tunnel lengths mined by larvae and ranked according to Duncan's new multiple range test for the first five days (Table VIII) give the following number of significant differences among these means in each group: .05 level - group I - 12, II - 5, III - 7, and IV - 14; .01 level - group I - 11, II - 5, III - 5, and IV - 11.

For the total duration of the test, an analysis of variance on the lengths of tunnels mined by larvae (Table VII) denotes that plant species within group I are not significantly different; plant species within group III are significantly different at the .05 level; and for groups II and IV, plant species are significantly different at the .01 level. Treatment means based on tunnel lengths mined by larvae and ranked according to Duncan's new multiple range test for the total duration of the test (Table VIII) give the following number of significant differences among the means in each group: .05 level - group I - 2, II - 5, III - 8, and IV - 17; .01 level - group I - 0, II - 5, III - 0, and IV - 11.

Test plant species rankings according to the host specificity index and the index of survivability are presented in Tables XII and

TABLE VII. F test results with degrees of freedom and standard error of mean based on tunnel lengths mined by larvae for both the first five days and total duration of the test.

	F [a]	df [b]	$s_{\bar{x}}$ [c]
First Five Days			
Group I	2.80*	26 & 12	5.21
Group II	6.35**	12 & 5	7.87
Group III	2.63*	22 & 10	4.69
Group IV	6.56**	18 & 8	3.64
Total			
Group I	1.57n.s.	26 & 12	46.15
Group II	6.01**	12 & 5	33.38
Group III	2.36*	22 & 10	5.98
Group IV	13.46**	18 & 8	5.20

a = F test
b = degrees of freedom
c = standard error of mean

TABLE VIII. Larval feeding based on mean tunnel lengths mined for the first five days and the total duration of the test with treatments ranked according to Duncan's new multiple range test.

	.05 [1/]	.01 [1/]
First Five Days		
Group I - Cynareae		
Cirsium arvense	a	a
Carduus acanthoides	ab	ab
Carduus nutans	ab	b
Cirsium vulgare	ab	b
Onopordum acanthium	abc	b
Carduus horridissimus	abcd	b
Xeranthemum annum	abcd	b
Carduus pycnocephalus	abcd	b
Silybum marianum	bcd	b
Centaurea montana	bcd	b
Centaurea nigrescens	bcd	b
Carlina acaulis	cd	b
Centaurea cyanus	d	b
Group II - Economic Composites		
Lactuca sativa	a	a
Carduus nutans	b	b
Cynara cardunculus	b	b
Cynara scolymus	b	b
Helianthus annus	b	b
Centaurea sultana	b	b
Group III - Other Composites		
Carduus nutans	a	a
Inula sp.	ab	ab
Helichrysum sp.	ab	ab
Taraxacum sp.	ab	ab
Senecio arenarius	b	ab
Chrysanthemum sp.	b	ab
Cichorium intybus	b	b
Tagetes erecta	b	b

1/ Means in a given row with similar letters are not significantly different according to Duncan's new multiple range test.

TABLE VIII (Continued). Larval feeding based on mean tunnel lengths mined for the first five days and the total duration of the test with treatments ranked according to Duncan's new multiple range test.

	.05 [1/]	.01 [1/]
Aster sp.	b	b
Cichorium endiva	b	b
Artemesia vulgaris	b	b
Group IV - Economic Plants		
Medicago sativa	a	a
Carduus nutans	a	ab
Brassica sp.	b	bc
Daucus carota	b	bc
Raphanus sativa	b	bc
Lycopersicon esculentum	b	c
Beta vulgaris	b	c
Zea mays	b	c
Capsicum frutescens	b	c
Total		
Group I - Cynareae		
Onopordum acanthium	a	a
Carduus acanthoides	ab	a
Xeranthemum annum	ab	a
Cirsium arvense	ab	a
Carduus nutans	ab	a
Cirsium vulgare	ab	a
Centaurea nigrescens	ab	a
Carduus horridissimus	ab	a
Silybum marianum	ab	a
Carduus pycnocephalus	ab	a
Centaurea montana	ab	a
Centaurea cyanus	b	a
Carlina acaulis	b	a

1/ Means in a given row with similar letters are not significantly different according to Duncan's new multiple range test.

TABLE VIII (Continued). Larval feeding based on mean tunnel lengths mined for the first five days and the total duration of the test with treatments ranked according to Duncan's new multiple range test.

	.05 [1]	.01 [1]
Group II - Economic Composites		
Lactuca sativa	a	a
Carduus nutans	b	b
Centaurea sultana	b	b
Cynara cardunculus	b	b
Cynara scolymus	b	b
Helianthus annus	b	b
Group III - Other Composites		
Carduus nutans	a	a
Helichrysum sp.	ab	a
Inula sp.	abc	a
Senecio arenarius	abc	a
Taraxacum sp.	abc	a
Tagetes erecta	abc	a
Chrysanthemum sp.	bc	a
Cichorium intybus	bc	a
Aster sp.	c	a
Cichorium endiva	c	a
Artemesia vulgaris	c	a
Group IV - Economic Plants		
Carduus nutans	a	a
Medicago sativa	b	b
Brassica sp.	bc	bc
Raphanus sativa	cd	bc
Daucus carota	cd	bc
Lycopersicon esculentum	cd	bc
Beta vulgaris	d	c
Zea mays	d	c
Capsicum frutescens	d	c

1/ Means in a given row with similar letters are not significantly different according to Duncan's new multiple range test.

TABLE IX. Summary of the primary host specificity test for the first experiment showing the parameters used in calculating the host specificity index (HSI) based on the standard: Carduus nutans (HSI = 100.00%).

	Mean # Days to Death of Last Larva	Total # Reaching 2nd Instar	Total # Reaching 3rd Instar	Mean Tunnel Per Rep (mm)	Mean Tunnel Per Larva (mm)	FI	HSI (%)
Group I (Cols.)	(1)	(2)	(3)	(4)	(5)	(1)(5)	
C. nutans	45.3*1	3	3	108.30	21.66	981.20	100.00
C. acanthoides	48.3	7	6	154.71	30.94	1494.46	470.15
C. pycnocephalus	6.6	0	0	23.17	4.63	30.58	9.62
C. horridissimus	13.3	0	0	31.86	6.37	84.75	26.66
S. marianum	8.3	0	0	25.62	5.12	42.53	13.38
C. arvense	60.0	5	3	113.40	22.68	1360.78	428.09
C. vulgare	46.6*1	3	2	106.96	21.39	996.87	313.61
C. acaulis	19.6	0	0	11.11	2.22	43.55	13.70
O. acanthium	37.0	4	2	174.93	34.99	1294.52	407.25
X. annum	31.3	2	0	119.41	23.88	747.52	235.17
C. montana	10.0	0	0	22.97	4.59	45.95	14.46
C. nigrescens	31.6	1	0	46.71	9.34	496.71	156.26
C. cyanus	11.6	0	0	14.87	2.97	34.50	10.85
Group II							
C. nutans	6.6*1	(1)	(1)	21.44	4.29	28.30	100.00
C. scolymus	5.0	0	0	16.67	3.33	16.67	5.24
C. carduncul us	5.0	0	0	17.68	3.54	17.68	5.56
H. annus	5.0	0	0	15.56	3.11	15.56	4.90
Lactuca sativa	48.6*1	5	1	218.66	43.73	2125.38	668.63
C. sultana	11.6	0	0	20.03	4.01	46.48	14.62

TABLE IX(Continued). Summary of the primary host specificity test for the first experiment showing the parameters used in calculating the host specificity index (HSI) based on the standard: Carduus nutans (HSI = 100.00%)

	Mean # Days to Death of Last Larva	Total # Reaching 2nd Instar	Total # Reaching 3rd Instar	Mean Tunnel Per Rep (mm)	Mean Tunnel Per Larva (mm)	FI	HSI (%)
(Cols.)	(1)	(2)	(3)	(4)	(5)	(1)(5)	
Group III							
C. nutans	5.0*3	1(1)	1(1)	28.50	5.70	28.50	100.00
Chrysanthemum sp.	10.0	0	0	7.94	1.59	15.88	5.00
S. arenarius	11.6	0	0	17.74	3.55	41.17	12.95
Aster sp.	6.6	0	0	5.36	1.07	7.07	2.22
Inula sp.	8.3	0	0	24.18	4.84	40.14	12.63
C. intybus	8.3	0	0	7.81	1.56	12.97	4.08
C. endiva	5.0	0	0	4.80	0.96	4.80	1.51
T. erecta	11.6	0	0	10.10	2.02	23.43	7.37
A. vulgaris	8.3	0	0	4.67	0.93	7.76	2.44
Helichrysum sp.	11.6	0	0	27.45	5.49	63.69	20.04
T. officinale	8.3	0	0	14.74	2.95	24.47	7.70
Group IV							
C. nutans	18.3*2	5	2	63.79	12.76	233.47	100.00
C. frutescens	8.3	0	0	5.00	1.00	8.30	2.61
L. esculentum	10.0	0	0	12.42	2.48	24.84	7.81
R. sativa	15.0	0	0	14.71	2.94	44.12	13.88
B. vulgaris	10.0	0	0	6.99	1.40	13.99	4.40
D. carota	8.3	0	0	13.01	2.60	21.59	6.79

TABLE IX(Continued). Summary of the primary host specificity test for the first experiment showing the parameters used in calculating the host specificity index (HSI) based on the standard: Carduus nutans (HSI = 100.00%)

Group IV (Cols.)	Mean # Days to Death of Last Larva (1)	Total # Reaching 2nd Instar (2)	Total # Reaching 3rd Instar (3)	Mean Tunnel Per Rep (mm) (4)	Mean Tunnel Per Larva (mm) (5)	FI (1)(5)	HSI (%)
B.o. botrytis	23.3	0	0	28.07	5.61	130.82	41.16
Zea mays	6.6	0	0	5.20	1.04	6.86	2.16
Medicago sativa	8.3	0	0	33.27	6.66	55.32	17.40

Legend: *Plant infested with 1, 2, or 3 larvae from subsequent re-infestation.
( ) Plant dissected and larvae found
Results based on three replicates with five inoculations per replicate.

TABLE X. Summary of the primary host specificity test after five days showing the parameters used in calculating the index of survivability (IS) based on the standard: *Carduus nutans* (IS = 100.0%)

	# Dead at 5 days	# cf at 5 days	# alive at 5 days	Body Length Growth (+)	(nc)	(-)
Group I						
C. nutans	0	4	11	11	0	0
C. acanthoides	5	3	7	6	0	6
C. pycnocephalus	2	12	1	0	1	2
C. horridissimus	1	9	5	1	0	4
S. marianum	2	11	2	1	0	3
C. arvense	2	6-(2)	7+(2)	6+(2)	0	2
C. vulgare	3	5	7	5	1	4
C. acaulis	10	0	5	1	0	10
O. acanthium	2	5	8	6*	1	3
X. annum	6	1	8	5	0	9
C. montana	10	1	4	1	1	12
C. nigrescens	4+1 ac	1	9	6**	0	7
C. cyanus	6+1 ac	2	6	2	1	9
Group II						
C. nutans	3	9	3	0	2	4
C. scolymus	7	7	1	0	0	8
C. cardunculus	10	5	0	0	0	10
H. annus	12	3	0	0	1	11
L. sativa	4	3	8	8	0	4
C. sultana	6	1	8	3	0	10

a-adjusted figure to account for exit and subsequent infestation.
b-adjusted figure to account for fact that despite decrease of body length growth, larva continued to survive and develop to 2nd instar.
* actually totals 7+
** actually totals 7+
1 Figures in parenthesis indicate that these plants appeared infested but subsequent dissection resulted in finding no larvae, although damage to the growing point was noted. Figures within the parentheses were not used in determining the IS standard mean figure of 1888.74 for *C. nutans*. Results based on three replicates with five inoculations per replicate.
cf= missing, nc=no change, ac=accidentally crushed.

TABLE X (Continued).

	# Dead at 5 days	# cf at 5 days	# Alive at 5 days	Body Length Growth (+)	(nc)	(-)
Group III						
C. nutans	2	13-(1)	0+(1)	0+(1)	0	2
Chrysanthemum sp.	8	0	7	3	0	12
S. arvense	6	1	8	5	0	5
Aster sp.	13	1	1	1	0	13
Inula sp.	6	1	8	3	0	11
C. intybus	10	0	5	2	0	13
C. endiva	9	6	0	0	0	8
T. erecta	9	2	4	1	0	11
A. vulgaris	12	1	2	1	0	14
Helichrysum sp.	5	1	9	2	0	12
T. officinale	7	1	7	1	0	12
Group IV						
C. nutans	2	5	8	6	0	3
C. frutescens	11	1	3	1	0	13
L. esculentum	3	0	12	0	1	14
R. sativa	0	6	9	2	0	7
B. vulgaris	9	1	5	0	0	12
D. carota	4	1	10	2	2	10
B. o. botrytis	2	2	11	7	4	1
Z. mays	13	0	2	2	0	13
M. sativa	12	1	2	1	1	12

TABLE X (Continued).

	Mean tunnel length Per Rep (mm)	Mean tunnel length Per Larva (mm)/5
Group I		
C. nutans	28.92	5.78
C. acanthoides	29.77	5.95
C. pycnocephalus	20.16	4.03
C. horridissimus	23.50	3.57
S. marianum	17.87	3.57
C. arvense	36.86	7.37
C. vulgare	28.63	5.72
C. acaulis	8.63	1.72
O. acanthium	25.23	5.04
X. annum	20.91	4.18
C. montana	16.44	3.29
C. nigrescens	15.10	3.02
C. cyanus	6.11	1.22
Group II		
C. nutans	21.11	4.22
C. scolymus	16.67	3.33
C. carduncululs	17.68	3.54
H. annus	15.55	3.11
L. sativa	63.99	12.80
C. sultana	10.13	2.03
Group III		
C. nutans	28.50	5.70
Chrysanthemum sp.	7.03	1.40
S. arvense	13.27	2.65
Aster sp.	5.36	1.07
Inula sp.	18.20	3.64
C. intybus	7.03	1.40
C. endiva	4.80	0.96
T. erecta	6.08	1.21
A. vulgaris	4.44	0.89
Helichrysum sp.	17.12	3.42
T. officinale	14.31	2.86
Group IV		
C. nutans	23.86	4.77
C. frutescens	4.38	0.87
L. esculentum	7.25	1.45
R. sativa	8.01	1.60
B. vulgaris	5.42	1.08
D. carota	8.86	1.77
B.o. botrytis	10.13	2.03
Z. mays	5.20	1.04
M. sativa	30.88	6.18

TABLE X (Continued).

	% Alive After 5 days	%With Body length growth	%Probable Survivability	Index of Survivability Standard C. nutans=1888.74
Group I				
C. nutans	73.33	73.33	5377.29	100.00
C. acanthoides	46.67	40.00	1866.80	98.84
C. pycnocephalus	6.67	0.00	0.00	0.00
C. horridissimus	33.33	6.67	222.31	11.77
S. mariamun	13.33	6.67	88.91	4.71
C. arvense	a60.00	53.33	3199.80	169.41
C. vulgare	46.67	33.33	1555.51	82.36
C. acaulis	33.33	6.67	222.31	11.77
O. acanthium	b53.33	46.67	2488.91	131.78
X. annum	53.33	33.33	1777.49	94.11
C. montana	26.67	6.67	177.89	9.42
C. nigrescens	b60.00	46.67	2800.20	148.26
C. cyanus	40.00	13.33	533.20	28.23
Group II				
C. nutans	20.00	0.00	0.00	100.00
C. scolymus	6.67	0.00	0.00	0.00
C. cardunculus	0.00	0.00	0.00	0.00
H..annus	0.00	0.00	0.00	0.00
L. sativa	53.33	53.33	2844.09	150.58
C. sultana	53.33	20.00	1066.60	56.47
Group III				
C. nutans	a 6.67	6.67	44.49	100.00
Chrysanthemum sp.	46.67	20.00	933.40	49.42
S. arvense	53.33	33.33	1777.49	94.11
Aster sp.	6.67	6.67	44.49	2.36
Inula sp.	53.33	20.00	1066.60	56.47
C. intybus	33.33	13.33	444.29	23.52
C. endiva	0.00	0.00	0.00	0.00
T. erecta	26.67	6.67	177.89	9.42
A. vulgaris	13.33	6.67	88.91	4.71
Helichrysum sp.	60.00	13.33	799.80	42.35
T. officinale	46.67	6.67	311.29	16.48
Group IV				
C. nutans	53.33	40.00	2133.20	100.00
C. frutescens	20.00	6.67	133.40	7.06
L. esculentum	80.00	0.00	0.00	0.00
R. sativa	60.00	13.33	799.80	42.35
B. vulgaris	33.33	0.00	0.00	0.00
D. carota	66.67	13.33	888.71	47.05
B. o. botrytis	73.33	46.67	3422.31	181.20
Z. mays	13.33	13.33	177.69	9.41
M. sativa	13.33	6.67	88.91	4.71

TABLE XI. Correlation of Logarithmic ranking between Host Specificity Index (Total) and the Index of Survivability (Five days).

	FIVE DAY (IS)					
	Very High	High	Moderate	Low	Very Low	TOTAL
TOTAL (HSI)						
Very High	4	3	0	0	0	7
High	1	1	0	0	0	2
Moderate	0	5	3	3	0	11
Low	0	2	2	1	6	11
Very Low	0	0	1	3	1	5
TOTAL	5	11	6	7	7	(36)

$XY = 355$

$X^2 = 390$

$Y^2 = 355$

$$r = \frac{XY}{(X^2)(Y^2)^{1/2}} = 0.9541$$

$t = 26.00^{**}$

TABLE XII. Test plant species ranked according to the host specificity index showing the feeding intensity (FU)(L) or feeding units times longevity of feeding and the number of larvae reaching the second and third instar. The index is based on the standard: *Carduus nutans* (HSI = 100%)

	(FU) (L) %	2nd Instar	3rd Instar
a. Most preferred Species (HSI > 100%)			
L. sativa	668.63	5	1
C. acanthoides	470.15	7	6
C. arvense	428.09	5	3
O. acanthium	407.25	4	2
C. vulgare	313.61	3	2
X. annum	235.17	2	0
C. nigrescens	156.26	1	0
b. HSI: High (30-100%)			
C. nutans	100.00	9(2)*	6(2)*
B.o. botrytis	41.16	0	0
c. HSI: Moderate (10-29.99%)			
C. horridissimus	26.66	0	0
Helichrysum sp.	20.04	0	0
M. sativa	17.40	0	0
C. sultana	14.62	0	0
C. montana	14.46	0	0
R. sativa	13.88	0	0
C. acaulis	13.70	0	0
S. marianum	13.38	0	0
S. arenarius	12.95	0	0
Inula sp.	12.63	0	0
C. cyanus	10.85	0	0

*Figures in parentheses indicate that two second and third instars were found to have re-infested the test plant.

TABLE XII (Continued). Test plant species ranked according to the host specificity index showing the feeding intensity (FU)(L) or feeding units times longevity of feeding and the number of larvae reaching the second and third instar. The index is based on the standard: Carduus nutans (HSI = 100%).

	(FU)(L)%	2nd Instar	3rd Instar
d. HSI: Low (3-9.99%)			
C. pycnocephalus	9.62	0	0
L. esculentum	7.81	0	0
T. officinale	7.70	0	0
T. erecta	7.37	0	0
D. carota	6.79	0	0
C. cardunculus	5.56	0	0
C. scolymus	5.24	0	0
Chrysanthemum sp.	5.00	0	0
H. annus	4.90	0	0
B. vulgaris	4.40	0	0
C. intybus	4.08	0	0
e. HSI: Very Low (< 3%)			
C. frutescens	2.61	0	0
A. vulgaris	2.44	0	0
Aster sp.	2.22	0	0
Z. mays	2.16	0	0
C. endiva	1.51	0	0

XIII. Results of a correlation between the two indices gives an $r = .95$ with a highly significant t value of 26.00.

Results of a second larval transfer test (Table XIV) indicated that C. horridissimus and C. nutans were infested to a greater degree than C. vulgare, C. acanthoides, and C. scolymus. While O. acanthium and H. annus did not support larval development, within O. acanthium a dead second instar was found, while plants of the latter species died and no second or third instar living or dead could be found.

TABLE XIII. Test plant species ranked according to the index of survivability (IS) showing the percent alive (%A) times the percent with a body length growth increase (%BLG+) and the feeding unit (FU) for the first instar larval transfer test after five days exposure to the test plants. The index is based on the standard: Carduus nutans (IS = 100%)

	(%A) (%BLG)	(FU)
a. Most probable survival: IS > 100%		
B. o. botrytis	181.20	2.03
C. arvense	169.41	7.37
L. sativa	150.58	12.80
C. nigrescens	148.26	3.02
O. acanthium	131.78	5.05
b. IS: High 30-100%		
C. nutans	100.00	5.12
C. acanthoides	98.84	6.00
S. arenarius	94.11	2.65
X. annum	94.11	4.18
C. vulgare	82.36	5.72
C. sultana	56.47	2.03
Inula sp.	56.47	3.64
Chrysanthemum sp.	49.42	1.41
D. carota	47.05	1.77
Helichrysum sp.	42.35	3.42
Raphanus sativa	42.35	1.60
c. IS: Moderate 10-29.99%		
C. cyanus	28.33	1.22
C. intybus	23.52	1.41
T. officinale	16.48	2.86
C. horridissimus	11.77	3.58
C. acaulis	11.77	1.72

TABLE XIII (Continued).

	(%A)	(%BLG+)	(FU)
d. IS: Low 3-9.99%			
C. montana	9.42		3.29
T. erecta	9.42		1.22
Z. mays	9.41		1.04
C. frutescens	7.06		0.88
A. vulgaria	4.71		0.89
M. sativa	4.71		6.18
S. marianum	4.71		3.58
e. IS: Very Low < 3%			
Aster sp.	2.36		1.07
B. vulgaris	0.00		1.08
C. pycnocephalus	0.00		4.03
C. endiva	0.00		0.96
C. cardunculus	0.00		3.54
C. scolymus	0.00		3.33
H. annum	0.00		3.11
L. esculentum	0.00		1.45

TABLE XIV. Summary of the second larval transfer test - duration 30 days, showing plant vitality, plant species infested, and the number of second and third instars infesting the test plant.

	Plant Vitality		Plant Infested		Second Instar		Third Instar		Total (RepsI & II)	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	2nd Instar	3rd Instar
C. horridissimus	L	L	Yes	Yes	0	1D	7	5	1D	12
C. nutans	L	L	Yes	Yes	0	0	4	7	0	11
C. vulgare	L	L	Yes	Yes	0	0	4	2	0	6
C. acanthoides	D	L	No	Yes	0	0	0	2	0	2
C. scolymus	L	L	Yes	Yes	0	0	1	1	0	2
O. acanthium	L	L	No	No	1D	0	0	0	1D	0
H. annus	D	D	No	No	0	0	0	0	0	0

L = Living

D = Dead

DISCUSSION

Before presenting a discussion of the results several clarifications should be made: 1) data are incomplete for Carduus acanthoides, Xeranthemum annum, and Centaurea cyanus since one larva in each of the replicates was accidentally crushed during the test and a substitute inoculation was not possible. Thus, any results of analyses for these three species are most probably underestimates of the actual trend with respect to the amount of mining, longevity, and development of the larvae; 2) Carduus pycnocephalus and Xeranthemum annum tests are also probably underestimates of an actual trend, since for these, some inoculated plants died during the latter aspects of the test and subsequently the cause of larval death in some cases could only be ruled as starvation. A short supply of both of these species limited the extent to which a successful test could be executed; 3) for some unknown reason, many larvae gained exit from their inoculation sites via exit mines and holes. This occurred in all plant species except five (Carlina acaulis, Chrysanthemum sp., Cichorium intybus, Lycopersicon esculentum, and Zea mays) and was most pronounced among the Cynareae. However, only among the Cynareae did subsequent reinfestation occur. This phenomenon may possibly hinder the significance of a test such as this, as well as the latitude to which this technique could be applied. The difficulties pertaining to the interpretation of the analysis and application of the results may result in potentially challengeable data which must be handled fortrightly and analyzed with care. An interpretation of the data

in light of these three clarifications should place the results in the proper perspective.

The results from the statistical analysis suggest some definite trends, however, with regard to the problem concerning larval escape from inoculation sites. The author feels that these analyses should not be interpreted without proper consideration of the indices. From the indices depicted in Tables XII and XIII, one observes that larvae exposed to a favorable test plant usually had a high percentage of survival and growth in the first five days of the test. In addition, these same larvae usually consumed a substantial amount of plant material and survived for a considerable amount of time on these plants, and subsequently developed to the second or third instar. This can readily be seen since only those plant species which supported larval development to the second or third instar are among those ranked as high or most preferred species in the host specificity index scale (Table XII). These same plants also scored among the most preferred and high in the index of survivability (Table XIII). Thus, from this perspective the following plants would be listed as potential host plants for first instars: Lactuca sativa, Carduus acanthoides, Cirsium arvense, Onopordum acanthium, Cirsium vulgare, Xeranthemum annum, Centaurea nigrescens, and Carduus nutans. Those plant species in which an antibiosis may be manifest include: Brassica oleracea botrytis, Carduus horridissimus, Helichrysum sp., Medicago sativa, Centaurea sultana, C. montana, C. cyanus, Raphanus sativa, Carlina acaulis, Silybum marianum, Senecio arenarius, and Inula sp.

The remaining plants ranking less than ten percent in the host specificity index scale could be considered from this experiment as being potential non-risk plants or non-hosts for C. horridus.

Before this conclusion can be drawn, if indeed it can even be stated, several relevant criticisms should be considered due to the nature of the above experiment. Handling of the larvae every five days could introduce a type of mechanical or handling error into the test and test results might be judged as underestimating the potential success of C. horridus on these plants. Those plant species which demonstrated an antibiosis and nonpreference effect may have been so borderline with respect to their acceptance by C. horridus that the sensitivity of the test may have been sacrificed by the periodic handling of the larvae. This could result in overlooking potential host plants.

This experiment also assumed that all plant material consumed by the larvae was of the same texture or form. This is a rather gross assumption which can explain, for example, the ratings of Brassica oleracea botrytis, Helichrysum sp., Medicago sativa since all of these species contain a light pithy inner stem which is consumed much more readily in comparison to the non-pithy portions of the inner stem. Since texture was not accounted for as a variable, it resulted in an overestimation of the degree of acceptance of the host species by virtue of "inflated" tunnel length figures.

With respect to the host specificity index and the index of survivability, two points should be clarified. First, it is not known

whether the parameters utilized in the formulas for determining the indices in the respective equations are significantly correlated with each other. The point is that non-significantly correlated parameters could offset the value of the index by tending to balance one parameter with the other while significantly correlated parameters would reflect their real value as intended in the indices by their correct additive effect. Secondly, the host specificity index equation does not accurately denote a true value of feeding intensity if a larva can survive on a test plant for a long period of time while feeding only moderately and yet not developing to a second instar. This is the case with B. o. botrytis (cauliflower). In such cases the index does not necessarily reflect an accurate trend for the test species.

Finally, in many cases larvae gained exit from their site of inoculation by boring exit tunnels through the plant cuticle. Only within the Cynareae did subsequent reinfestation occur. Due to this behavior many larvae were able to survive and consume plant material, but no measurements of tunnel length or larval longevity could be made since symptoms were often not noticeable for two weeks or more. Thus, for those plants which experienced this phenomenon and later became infested (Carduus nutans and Cirsium vulgare), results are definitely an underestimate of their potential as host plants. Since C. nutans was used as a control plant in each of the four groups of plants tested, both the statistical analysis and the indices approach of ranking the test plants would not necessarily give true figures.

Instead, only potential trends can be depicted from the results, thus sacrificing quantitative interpretations in deference to qualitative interpretations.

Results from the second larval transfer test are indicative of the HSI scale rankings with the exception of C. scolymus and C. horridissimus which ranked low and moderate respectively; all other plants tested reflected their previous rankings. The best technique of determining larval acceptance and infestation potential is to design temporal tests; plant species inoculated would be dissected and inspected once in time bound sequence sets of possibly 10 days between check points. Attempts to reinoculate a plant with a larva involves extra risk of using a damaged larva, while dissection of only a portion of an infested plant to determine feeding damage involved the risk of missing a portion of the damage.

A. Discussion of Economic Plants, Cynareae, and Other Species

Since one of the purposes of these tests is to determine the insect's effect upon several important economic plants, results will be discussed with respect to Cynara scolymus, Lactuca sativa, Brassica oleracea botrytis, and Carthamus tinctorius. In addition, results will be analyzed for the tribe Cynareae and for the other species of plants tested but not included in other discussions.

Economic Plants

Cynara scolymus, Artichoke

C. scolymus, a member of thr tribe Cynareae, subtribe Carduinae, is an important economic plant. The genus Cynara is closely related

to the genera Carthamus, Centaurea, and Cirsium (Munz, 1959). It is believed to be derived from the wild species Cynara cardunculus (Brecane and Artigas, 1932). Earliest records date back to the naturalist Theophrastus (371-287 B.C.) who reported artichokes being grown in Italy and Sicily (Scammel, 1970). The cultivated artichoke is a perennial lasting four to seven years, blooming in warm climates, from early winter to April. Due to the close relationship between these two species of Cynara, they are often both included in testing programs. As stated previously both ranked low in the HSI scale, but of thirty inoculated into the midrib, C. scolymus supported the development of two larvae to the third instar. Whether completion of a life cycle could be attained is still not known. Dunn (1970c) reported a moderate amount of feeding on artichoke (79mm^2 / adult) and C. cardunculus (215 mm^2 / adult) with four adults per test plant. Dunn observed after 11 days, 73 and 159 eggs laid on artichoke and cardoon respectively, and the adults survived 155 and 249 days respectively. He observed oviposition after an arbitrary time period of 11 days, since the first eggs were thought to be remnants of those produced from the Carduus feeding. A similar test conducted by the author[2] with C. scolymus with 5 males and 5 females per test plant

[2] This experiment was begun on November 8, 1971 with all cages of adults having terminated diapause by this time. However, the time of diapause termination varied considerably among the cages because each treatment was not exposed to the same precondition criteria (photoperiod and temperature regime) which was in accordance with the prescribed procedures for the diapause termination experiment. For this reason adults used in the experiment had been out of diapause from 1 - 67 days prior to the initiation of the adult starvation and oviposition experiment. Subsequently, this data provides only a relative indication of the amount of feeding and oviposition under these conditions.

resulted in a moderate amount of feeding, with 14 eggs oviposited on the cotton wrapping the midrib and the males still alive after 187 days. From these data it is now important to determine whether the larvae can successfully develop to adults on these two species. If this is the case, it is doubtful that C. horridus will be recommended for release, unless a chemically defined repellent, suppressant, or deterrent can be shown to be the active agent which protects C. scolymus from attack in the field. Sherf (1964) has not reported C. horridus as being a pest of either artichoke or cardoon.

Lactuca sativa, Lettuce

Lactuca sativa, a member of the tribe Liguliflorae of the Compositae family consists of varieties which include three classes: butter, crisp, and Cos, and has been under cultivation for 2500 years. Larval tests indicated that lettuce ranked highest among the most preferred plant species and was significantly different from all other species within Group II at the .01 level for both the first five days test and for the total experiment. Larval development proceeded to the third instar and longevity exceeded 109 days. However, Dunn (1970c) reported no adult feeding or oviposition on lettuce and the adults lived only 22 days. A similar study [3] indicates that feeding is minimal and usually occurs on the cut leaf edges with oviposition consisting of 2 eggs laid within the first nine days, and longevity exceeding 187 days with 2 males still alive. Harris and Zwolfer (1968)

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[3] See footnote 2, page 69.

pointed out that the leaves of lettuce are bland, lacking both attractants and deterrents, and thus forming a relatively neutral substrate which is accepted by many stenophagous Coleoptera since it is a source of moisture and essential nutrients. Also, the fact that adult feeding and oviposition are minimal under confined conditions, and that C. horridus has never been reported as a pest of lettuce, this insect should not pose a problem in lettuce fields.

Brassica oleracea botrytis, Cauliflower

This species is a member of the Cruciferae and is an economically important vegetable. Cruciferous plants are attacked by many species of the related genus Ceutorhynchus and it has been reported that the larvae of Ceuthorrhynchidius sp. may possibly inhabit Cruciferae (Sherf, 1964). Since this species of Brassica ranked high on the HSI scale and larvae survived a mean of 23.3 days with a fair degree of mining of the plant, and also ranked the highest of any species in the index of survivability, this species may raise some concern. Is this simply a case of antibiosis, tolerance, or an artifact of the experimental technique? Additional larval and adult studies are in order not only for this species but perhaps several other members of the Cruciferae.

Carthamus tinctorius, safflower

Unfortunately this species was not included in this testing program. It is a very important economic plant of the tribe Cynareae, subtribe Ceutaureinae. A study of the fruit anatomy of 20 species of the ill-defined genera Carthamus and Carduncellus

indicated that the anatomical features of the achenes disclose a close relationship of this complex with the genera Serratula L. and Centaurea L. (Dittrich, 1969). An earlier investigation of some species of Carthamus for their polyacetylene compounds (polyines) also indicated this genus to be closely related to the section Centaurea (Bohlman et al., 1966).

Within the Carthamus genus, species are assigned to four sections based on chromosome numbers and morphological characteristics. C. tinctorius belongs to Section I, with 12 pairs of chromosomes and is closely related to C. oxyacantha M.B. and C. palaestinus E.G. (Ashiri and Knowles, 1960). However, from inheritance studies in interspecific hybrids of C. flavescens and C. tinctorius, it is indicated that the former, an annual herbaceous weed, indigenous to the Middle East, played a role in the evolution of the cultivated safflower, C. tinctorius (Imrie and Knowles, 1970).

C. oxyacantha, also possibly another wild ancestor of C. tinctorius is the most important noxious weed of winter crops, especially cereals, in many parts of West Pakistan (Mohyuddin et al., 1965). The genus Carthamus, containing about 25 species, has a natural distribution in Eurasia and Africa from the Mediterranean region, the Nile Valley to Ethiopia, Southwestern Asia and parts of Pakistan and India (Mohyuddin et al., 1965). Of these 25 or so species, only C. tinctorius is cultivated, while several of these species have been accidentally introduced into California, Argentina, South Africa, and Australia (Mohyuddin et al., 1965).

These relationships within the subtribe and genus all seem to indicate that definitive studies are necessary perhaps not only with the evolved cultivated species but also with its ancestors, since the latter were more than likely present when the ancestor species to C. horridus and its sibling species C. urens evolved on the Carduinae.

If it is found that C. horridus does attack the weedy Carthamus species in the Middle East and that these same weed species occur in the Western United States, then it is doubtful that C. horridus could be introduced simply on the basis that C. tinctorius is possibly a hybrid of these weed species and by means of back-crossing to the cultivated variety susceptible varieties of the cultivated varieties could result despite the fact that C. horridus may not attack cultivated safflower. Furthermore, from a genetic point of view, if the ancestral weevil species of C. horridus and C. urens could attack the ancestral safflower species, then either one or both weevils may contain the proper germplasm to initiate this behavioral pattern once again if introduced near cultivated safflower, even if the cultivated varieties are presently outside their normal geographical range. This could result from the hybridization process mentioned as well as from the possession of ancestral germ-plasm by either sibling species.

As Schoonhoven (1968) pointed out, however, it is often the ovipositing adult rather than the larva which performs the actual host selection. Since C. horridus eggs and larvae are endophytic in host plant leaf midribs, and developing larvae mine toward the

meristematic tissue, the oviposition site is the means by which this insect's selection of its host is manifested. Dunn (1970c) showed that adults laid no eggs on safflower in an adult starvation and oviposition trial. The present study[4] using 5 males and 5 females per test substantiated Dunn's results, although 5 eggs were oviposited on the cotton wrapping the plant stem, and a moderate amount of adult feeding was reported supporting the adults for a total of 42 days. In addition, field reports (Zwolfer, 1965b; Avidov and Kotter, 1966) indicated that C. horridus does not attack this species. However, it has been reported that C. horridus was associated with the genus Carthamus (Schaufuss, 1916). Larval testing may be essential to verify that development cannot occur.

Cynareae

One aspect of the larval testing program was to define a larval host range among the tribe Cynareae with respect to C. horridus and to determine its potential for growth and development within the tribe. Data from Table VIII does not adequately depict this concern; however, data from Tables XII, XIII, and XIV does approach this definitive relationship. It appears that this range may encompass the subtribes Carlininae (Xeranthemum annum), Carduinae (Carduus sp.., Cirsium sp., Onopordum sp., and Cynara sp.) and the Centaureae (Centaurea sp.). This corresponds to some degree with adult starvation tests previously studied by Zwolfer and Harris (1966) and Zwolfer (1972b). Feeding was most pronounced on Cirsium - Silybum - Carduus

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[4] See footnote 2, page 69.

group, with some nibbling on Onopordum, and with feeding also occurring on some species of Centaurea and to a lesser extent on Cnicus, Xeranthemum, and Aster. Dunn (1970c) reported significant feeding and oviposition on Carduus, Cirsium, Cynara, and Galactites while light oviposition with no feeding occurred on Centaurea cineraeia, Cirsium monspessulanum, Carduus pycnocephalus, Helianthus annus, Beta vulgaris, Daucus carota, and Chrysanthemum sp. In a similar adult feeding and oviposition test[5], significant feeding and oviposition occurred on Carduus nutans, C. acanthoides, and Cirsium vulgare, while light feeding and oviposition have been noted for the remaining species Helianthus annus, Cynara scolymus, Lactuca sativa, and Carthamus tinctorius. Field reports revealed that C. horridus is associated with Carduus nutans, C. pycnocephalus, Galactites tomentosa, Cirsium arvense, and C. lanceolatum in Italy and Carduus acanthoides, C. crispus, and Onopordum acanthium in Europe (Coulson, 1968).

The field reports show a much narrower range of plants than is suggested by laboratory tests which is natural since the insect is usually specialized in many features of its biology, morphology, phenology, ecology, physiology, and ethology to its host (Zwolfer and Harris, 1971). Some of those features which in a gravid female may be operative in the mechanism of host selection usually become inoperative in a starvation or negative oviposition test since confinement disrupts the normal progression of orienting responses

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[5] See footnote, 2, page 69.

to the series of evoking stimuli "emitted" by the host plant. Host plants are determined by the presence and recognition of stimuli and the absence of inhibitory stimuli (Zwolfer and Harris, 1966, 1971). These stimuli whether they be chemical or physical seem to coincide with the fact that cytomorphological similarities exist among Carduus, Cirsium, Cnicus, Onopordum and possibly Silybum, and that these genera are evolutionarily less divergent as a group than other closely related genera (Moore and Frankton, 1962). What is suggested is that C. horridus may recognize a number of species within the tribe Cynareae as host plants, but through selection processes such as niche exclusion, geographical isolation, spatial isolation, seasonal isolation, etc., it has evolved confinement to a host range limited to certain plant species which it comes to "recognize" with a high degree of specificity.

This aspect of behavioral responses of insects to host plant finding and acceptance stimuli is considered to be more stable from an evolutionary standpoint than is physiological adaptation to a food (Mayr, 1958) as pointed out by Zwolfer and Harris (1971). Zwolfer and Harris then presented the basic problem of all host specificity testing, that being: "The rigidity of behavior and relative plasticity of physiological adaptation make it desirable for host specificity determination of weed insects to be based primarily on an understanding of the host selection mechanism rather than on the results of starvation tests." This seems to be the direction in which many researchers are headed. Is it not more efficient to dig about the

root than to flail about the branches when supportive mechanisms are being sought?

Other Plant Species

With regard to the other plant species tested in each of the groups which have not been discussed, these being: Helichrysum sp., Medicago sativa, Centaurea sultana, Raphanus sativa, Senecio arenarius, Inula sp., Lycopersicon esculentum, Taraxacum officinale, Tagetes erecta, Daucus carota, Chrysanthemum sp., Helianthus annus, Beta vulgaris, Cichorium intybus, Capsicum frutescens, Artemesia vulgaris, Aster sp., Zea mays, and Cichorium endiva, these seem to pose no problem since larvae were short-lived, the HSI figures were below 21%, and no larva developed to second instar on any of these species. Since the larvae were not able to tolerate these species, then they might be included under Painter's (1951, 1958) definitive headings for plant resistance of "non-preference" and antibiosis. However, the problems associated with the primary host specificity experiment and the HSI and IS indices should be considered more thoroughly before these test plants can be entirely discounted as potential host plants.

RESULTS OF LARVAL MEASUREMENTS

To date no morphological description of the larva has been undertaken. Sherf (1964) indicated that C. horridus in Europe occurs in the stems of Onopordum acanthium along with Apion onopordi, Cleonus piger, and Lixus piger. C. horridus and A. onopordi can be differentiated from the latter two by body length since these are not over 5 mm long, while the latter two species are over 5 mm long. It is yet still not possible to differentiate C. horridus from A. onopordi. Since larval instar is not indicated, it is possible for all to be of equal length at a particular instar. Thus, it seems that taxanomic differentiation necessitates the need for morphological descriptions.

To clarify the measurements for C. horridus, head capsule and body length measurements for all three instars are presented in Table XV.

Table XVI gives the larval growth statistics for head capsule width and body length for C. horridus on five different species of plants at the end of 30 days.

Tables XVII and XVIII illustrate larval growth on eight species of plants for the primary host range experiment.

TABLE XV. C. horridus larval measurements showing the number of measurements (N), the mean, confidence limits, and range for both the head capsule and body length measurements.

	N	$\bar{X} \pm CL$	RANGE
Head Capsule Width (mm)			
First instar	869	0.279 ± 0.002	0.245 ± 0.298
Second instar	30	0.390 ± 0.003	0.350 ± 0.420
Third instar	51	0.525 ± 0.015	0.525 ± 0.718
Body Length (mm)			
First instar	869	1.236 ± 0.003	0.805 ± 1.452
Second instar	30	2.321 ± 0.167	1.575 ± 3.325
Third instar	51	5.055 ± 0.323	1.961 ± 6.765

TABLE XVI. Third instar measurements for the second infestation experiment at the end of 30 days showing the number of measurements (N), head capsule width, and body length for five species of plants.

	N	HEAD CAPSULE WIDTH $\bar{X} \pm$ CL (mm) (0.05)	BODY LENGTH $\bar{X} \pm$ CL (mm) (0.05)
C. nutans	11	0.609 ± 0.035	5.570 ± 0.433
C. acanthoides	2	0.595 ± 3.321	5.294 ± 1.266
C. horridissimus	12	0.653 ± 0.025	5.730 ± 0.426
C. vulgare	6	0.604 ± 0.059	5.588 ± 0.730
C. scolymus	2	0.595 ± 3.321	5.882 ± 2.953

TABLE XVII. C. horridus growth data for the primary host specificity experiment showing the mean number of days, mean tunnel length, mean head capsule width and body length for the first instar. (Measured in mm)

	Number of Measurements	Mean # Days to 2nd Instar	Mean Tunnel Length mined to 2nd Instar	Mean Head Capsule Width of 2nd Instar	Mean Body Length After Molting to Molting to 2nd
C. nutans	5	11-	25.24	0.396	2.222
C. acanthoides	5	11-	24.20	0.402	2.961
C. arvense	3	10-	24.18	0.391	2.412
C. vulgare	2	15-	26.27	0.385	1.768
O. acanthium	2	17.5-	30.20	0.402	2.012
X. annum	2	15.0-	31.42	0.385	2.135
C. nigrescens	1	20-	21.27	0.350	-
L. sativa	5	11-	35.32	0.374	2.100

TABLE XVIII. C. horridus growth data for the primary host specificity experiment showing the mean number of days, mean tunnel length, mean head capsule width and body length for the second instar. (Measured in mm)

	Number of Measurements	Mean # Days to 3rd Instar	Mean Tunnel Length Mined to 3rd Instar	Mean Head Capsule Width of 3rd Instar	Mean Body Length Molting to 3rd
C. nutans	2	15-	41.96	.569	3.158
C. acanthoi-des	3	16.25-	35.44	.578	4.409
C. arvense	0	-	-	-	-
C. vulgare	1	35-	64.61	.525	2.588
O. acanthium	1	35-	169.90	.525	2.176
X. annum	0	-	-	-	-
C. nigrescens	0	-	-	-	-
L. sativa	1	25-	143.53	.578	4.804

DISCUSSION OF LARVAL MEASUREMENTS

Information in Table XVI seems to indicate little difference between the head capsule and body length measurements for the five species of plants tested. Since the size of plants tested was not kept constant and the number of measurements (N) were small for some plant species, a statistical analysis of the data was not feasible.

Data from Tables XVII and XVIII indicate some differences between the head capsule and body length measurements, the tunnel length mined, and the number of days required for instar development for the eight plant species tested. Since some data were excluded because larval escape from inoculation sites prevented accurate day to day measurements to be taken and subsequently the number of measurements (N) were small for all species, a statistical analysis of these data was not feasible. Branson and Ortman (1967a, 1970) were able to determine from adult head capsule and body weight measurements for the western corn rootworm, Diabrotica virgifera LeConte that there was a "nutritive effect" upon this insect within its host range. The effect was expressed in terms of significantly smaller adults being reared out from some hosts as compared with other host plants. The author believes that a similar effect may be implicit from the C. horridus data presented in Tables XVII and XVIII. If this is the case, it may be indicative of a subtle host specificity mechanism operating in an evolutionary sense upon the weevil. If these "smaller"

larvae require a longer time period to mature, and are shown to be less viable by virtue of having been reared upon substandard hosts, and the adults from such larvae, though highly mobile, are found to remain upon the host from which it was reared, it may be possible to demonstrate that selection pressures are operative in maintaining the species' integrity toward its most preferred and nutritionally suited host plants.

RESULTS OF OVIPOSITION OBSERVATIONS

The females oviposited on the leaf, the cotton wrapping the stem, and in the stem of the Carduus nutans leaves provided. Eggs were found from the base of the midrib to the tip of the leaf oviposited singly or in clumps as great as 12, but usually in clumps of 3 - 5 per cavity. In the midrib eggs were found in deep, hollowed out cavities which were exposed to the outside by a minute hole in the midrib cuticle. These cavities differed from feeding cavities since the latter were much more exposed to the outside by removal of portions of the cuticle by the insect leaving holes often as large as the cavity itself. Most eggs were found surrounded by at least a film of water, while those oviposited at the leaf tips were dry and in all cases failed to hatch when placed in the egg containers.

In a starvation, feeding and oviposition experiment with seven[6] species of plants, females oviposited in the midribs of Carduus nutans, C. acanthoides, and Cirsium vulgare, but failed to oviposit on Helianthus annus, Carthamus tinctorius, Lactuca sativa, and Cynara scolymus. However, oviposition was observed on the cotton wrapping the leaf in the latter three species, C. tinctorius - 5 eggs, Lactuca sativa - 2 eggs, and Cynara scolymus - 14 eggs.

An analysis of egg hatch time from the feeding and oviposition experiment showed that of the 435 egg hatches analyzed, eggs hatched

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6 See footnote 2, page 69.

a mean of 11.9 days after being placed in the incubation cups (Table XIX). Since the experiment was checked every three days and it is possible for an egg to have been oviposited three days prior to being checked, the confidence limit appears to fall somewhere within $\pm$ 2 days. Thus, hatch mean time from oviposition would then be 13.4 days $\pm$ 2 days. The hatch time in the incubation cups ranged from 8 to 21 days.

Table XX presents the egg data information regarding the number oviposited, the number and percent which succumbed to various causes, and the number and percent of the eggs which hatched.

TABLE XIX. Number of days to hatch from day of placement into egg incubation cups and number of eggs hatched for C. horridus.

DAY	NUMBER OF EGGS HATCHED	NUMBER OF EGGS BY DAYS TO HATCH
1-7	0	0
8	17	136
9	2	18
10	79	790
11	63	693
12	106	1272
13	134	1742
14	14	196
15	15	225
16	3	48
17	1	17
18	0	0
19	0	0
20	0	0
21	1	21
TOTAL	435	5148

$$\text{Weighted mean} = \frac{5148}{435} = 11.9 \text{ days}$$

Approximately 91% of the eggs hatched within $\pm$ 2 days of the mean hatch time of 11.9 days.
Therefore: egg hatch mean time is approximately 11.9$\pm$ 2 days.

TABLE XX. C. horridus egg data giving the total number oviposited and the number and percent succumbing to various causes.

	NUMBER	PERCENT
Total laid	2346	100.0
Number crushed	41	1.7
Fungus	314	13.3
Sterile	82	3.5
Unaccounted for	108	4.6
Rotted in stem	142	6.0
Hatched	1659	70.5

DISCUSSION OF OVIPOSITION OBSERVATIONS

Dunn (1970c) reported the oviposition from an adult starvation feeding and oviposition trial as follows, for oviposition occurring eleven days after the initiation of the test: Galactites tomentosa - 269 eggs, Carduus acanthoides - 170 eggs, Cynara cardunculus - 159 eggs, Cirsium lanceolatum - 91 eggs, Cynara scolymus - 73 eggs, Carduus nutans - 20 eggs, Daucus carota - 12 eggs, Carduus pycnocephalus 9 eggs, Helianthus annus - 4 eggs, Cirsium monspessulanum and Centaurea cineraria - 3 eggs each, and Chrysanthemum sp. and Beta vulgaris - 1 egg each.

These results vary considerably from those found by the author. The reason is believed to arise from the fact that the gravid females used by the author varied considerably in reproductive status. At the initiation of the experiment, some females had just terminated diapause while others had terminated diapause up to 67 days prior to the beginning of the experiment.

From Table XX it should be noted that percent hatch could be adjusted upward by including the categories: number crushed, number unaccounted for, and the number rotted in the stem. This would result in approximately 1,950 eggs hatched and a 82.8 percent hatch.

SUMMARY AND CONCLUSIONS

From the larval transfer tests, the host specificity of the first instar was determined among 28 species of composites, 6 species of vegetables, and 2 forage species. In addition, the host suitability for C. horridus larvae was determined for 7 species of plants from a second larval transfer test. Plants were divided into four groups, the Cynareae, economic composites, other composites, and economic plants. Results of these experiments may be summarized as follows:

1. C. horridus larvae were able to develop to the third instar on the following economic plants: Lactuca sativa and Cynara scolymus.

2. C. horridus larvae were able to develop to the third instar on the following weed thistles: Carduus nutans, C. acanthoides, C. horridissimus, Cirsium arvense, C. vulgare, and Onopordum acanthium.

3. C. horridus larvae were able to develop only to the second instar on Centaurea nigrescens and Xeranthemum annum.

4. C. horridus larvae were able to survive for 25 days or more on the following plant species: Carduus nutans, C. acanthoides, C. horridissimus, Cirsium arvense, C. vulgare, Centaurea nigrescens, Carlina acaulis, Xeranthemum annum, Onopordum acanthium, Cynara scolymus, Lactuca sativa, and Brassica oleracea botrytis.

5. An analysis of variance on the lengths of the tunnels mined by larvae reveals that plant species within groups I and III are significantly different at the .05 level for the first five day period while plant species within groups II and IV are significantly

different at the .01 level. For the total duration of the experiment, plant species within group I are not significantly different, while those in group III are significant at the .05 level and those in groups II and IV are significant at the .01 level.

6. Treatment means based on tunnel lengths mined by larvae and ranked according to Duncan's new multiple range test for the first five days of the experiment give the following number of significant differences among the means in each group: .05 level - group I - 12, II - 5, III - 7, and IV - 14; .01 level - group I - 11, II - 5, III - 5, and IV - 11; for the total duration of the experiment: .05 level - group I - 2, II - 5, III - 8, and IV - 17; .01 level - group I - 0, II - 5, III - 0, and IV - 11.

7. From a larval host specificity index as defined in this study, the following plant species were determined to be among the highly or most preferred species: Lactuca sativa, Carduus acanthoides, Cirsium arvense, Onopordum acanthium, Cirsium vulgare, Xeranthemum annum, Centaurea nigrescens, Carduus nutans, and Brassica oleracea botrytis.

8. From a larval index of survivability as defined in this study, the following plant species were determined to be among the highest or most probable to support the larva after five days exposure to the plant: Brassica oleracea botrytis, Cirsium arvense, Lactuca sativa, Centaurea nigrescens, Onopordum acanthium, Carduus nutans, C. acanthoides, Senecio arenarius, Xeranthemum annum, Cirsium vulgare, Centaurea sultana, Inula sp., Chrysanthemum sp., Daucus carota, Helichrysum sp., and Raphanus sativa.

9. Before any decision can be based on these results as to which plant species can be defined as the host range for the first instar of C. horridus, the discussion of the host specificity testing program should be consulted so that the problems and difficulties concerning this testing program can be carefully considered and weighed accordingly.

II. AESTIVAL DIAPAUSE TERMINATION

INTRODUCTION

Diapause is an important adaptive mechanism for insect survival during periods of unfavorable environmental conditions such as extreme summer heat and dry periods. Records indicated that C. horridus undergoes an aestival diapause in the southern areas of its distribution (Rome, Italy) and a hibernal diapause in the northern areas of its distribution (central and northern France). Since the population of C. horridus used in this study was introduced from Rome, two major questions regarding the nature of the population are of importance: first, its potential to adapt to the climate of Virginia, and secondly, the potential for culturing and mass rearing this weevil. With these questions in mind, an experiment was designed to determine the photoperiod and temperature regime which could be most effective in terminating the aestival diapause of teneral adults.

LITERATURE REVIEW

A. Diapause and Diapause Induction

Diapause is defined as a genetically determined state of suppressed development (Beck, 1968). This "dormant state" may occur at any growth stage of an insect's development, but usually in accord with the particular environmental circumstances to which a species or geographical population is exposed, and which threatens its existence to the extent that strong genetic selection occurs. These environmental circumstances may be climatic such as low winter

temperatures, extreme heat, periods of dryness; or may be related to the periodic scarcity of food such as the seasonal disappearance of a specific botanical host phenophase as pointed out by Danilevskii (1961). For these reasons, Danilevskii indicated that the adaptations of diapause stages to climatic factors show greater specificity, and are more varied and complex than adaptations in the active stages.

As the adaptive mechanism suggests, insects can either undergo a hibernal or an aestival diapause. Relatively few species are known to be capable of either at more than one point in their life cycle. Steinberg and Kamensky (1936) introduced the terms facultative and obligatory to describe the utility of diapause in multivoltine and univoltine insect populations respectively. The facultative condition indicates that in polycyclic species the potential for diapause may not be manifested in a given individual or population in each generation indicating that the environmental conditions prevailing during certain critical stages of the insect's development dictate the necessity for diapause. Obligatory diapause denotes that regardless of the environmental conditions prevalent during the critical stage every individual of every generation enters diapause at a definite stage in each generation. This type of diapausing condition is normally feasible only for monocyclic or univoltine species.

In terms of the photoperiodic induction of diapause, a number of terms are commonly used. Critical day length is the point of

transition between a photoperiod triggering a very high and very low incidence of diapause. The incidence of diapause is a frequent expression used in association with diapause response and is plotted as a diapause induction response curve. There are four types of diapause induction curves as pointed out by Beck (1968). The type I or long-day response, type II or short-day response, type III or the short-day - long-day response, and type IV or the long-day - short-day response. The type II response which is pertinent to this study indicates that short daylengths favor the nondiapausing condition while relatively high temperatures tend to induce diapause and low temperatures promote the nondiapausing state.

The specific relationship of photoperiod, temperature, light intensity, light quality, nutrition, moisture and other factors upon diapause induction is quite complex. It is generally believed that photoperiod is of the greatest importance although the effects of photoperiod have been found to be modified or even nullified by the other factors.

B. Diapause Termination

C. horridus apparently displays the type II, since it was introduced from Rome. This type is discussed by Lees (1955), Danilevskii (1961), and Beck (1968). Examples of insects demonstrating the type II diapause are presented in Table XXI.

Of the species given in the table, Stenocranus minutus, Limnephilus spp., Hypera postica, Ceutorhynchus pleurostigma, and Longitarsus jacobaeae, all exhibit an adult aestival diapause.

TABLE XXI. Insect species for which a type II or short day response has been demonstrated or reasonably inferred in some geographical populations.

Order and Family	Genus and Species	Diapause Stage	References
Lepidoptera			
Noctuidae	Agrotis triangulum	(not known)	Danilevskii (1961)
	Mamestra brassicae (L.)	pupal	Masaki & Sakai (1965)
Arctiidae	Parasemia plantaginis (L.)	larval	Danilevskii (1961)
Lymantridae	Dasychira pudibunda L.	larval	Geyspitz (1953)
Geometridae	Abraxas miranda Butler	pupal	Masaki (1957a, 1958, 1959)
Saturniidae	Antheraea pernyi Guer	pupal	Danilevskii & Geyspitz (1948)
Bombycidae	Bombyx mori (L.)	embryonic	Kogure (1933)
Coleoptera			
Chrysomelidae	Longitarsus jacobaeae (Waterhouse)	adult	Frick & Johnson (1972)
Curculionidae	Hypera postica (Gyll.)	adult	Guerra & Bishop (1962) Huggans & Blickenstaff (1964)
	Ceuthorhynchus pleurostigma (Marsham)	adult	Ankersmit (1960)

TABLE XXI. Insect species for which a type II or short day response has been demonstrated or reasonably inferred in some geographical populations. (Continued)

Order and Family	Genus and Species	Diapause Stage	References
Homoptera			
Delphacidae	Stenocranus minutus	adult	Muller (1957, 1958, 1960)
Trichoptera			
Limnephilidae	Limnephilus spp.	adult	Novak & Schnal (1963)

For some of these species diapause termination was achieved by means of specific procedures and methods. S. minutus displays a rather complex realtionship to photoperiod. Long-day reared adults enter an aestival diapause which could be terminated after four weeks exposure to short days. Developing nymphs reared under similar short-day conditions resulted in adults which diapause and this could be terminated by a three week exposure to long days followed by a return to short-day photoperiods (Muller, 1958, 1960a).

Limnephilus spp. which normally aestivate as adults were found to terminate diapause upon an exposure to short-day photoperiods (Novak and Schnal, 1965).

H. postica likewise aestivates as an adult upon pupal eclosion in the late spring. In a series of experiments by Huggans and Blickenstaff (1964), results indicated that adult diapause is photoperiodically controlled by the larval exposure in the field, but successive laboratory reared generations of larvae lose this control over diapause induction in the adult, yielding generations which terminate diapause within a month or a month and one-half after pupal eclosion regardless of photoperiod length from 6 to 14 hours. The authors stated that this phenomenon was probably achieved by unintentional selection for nondiapausing weevils.

The autumn race of C. pleurostigma also aestivates as an adult upon pupal eclosion in June in the Netherlands. Ankersmit (1964, 1965) found that teneral adults exposed to long day photoperiods and relatively high temperatures entered aestival diapause with

critical daylength occurring between 14 and 16 hours and temperatures above 21°C. Diapause termination occurred with daylengths shorter than the critical daylength and with temperatures between 17° and 21° C. Ankersmit (1960) indicated that aestival diapause for this species does not occur if teneral adults are exposed to a short photoperiod.

The Italian biotype of L. jacobaeae experiences an aestival diapause as an adult while the Swiss biotype aestivates in the egg stage (Frick and Johnson, 1972). Upon exposure to daily regimen of 12 hours of light (24 ± 1°C) and 12 hours of dark (12.75 ± 1°C) 85 percent of the Italian eggs (from Rome) hatched within 3 weeks while the Swiss eggs (from Delemont) entered aestival diapause and took 15 weeks for 50 percent eclosion. By unintentional selection of progeny from early hatching Swiss eggs, the length of diapause during 8 generations (4 years) dropped from 17 weeks to 6 weeks.

MATERIALS AND METHODS

Information regarding the rearing of adults, cage specifications, and techniques of handling eggs are provided in the preceding methods and materials section. For this study, the following information was recorded throughout the experiment at each provision of fresh musk thistle leaves: date, male and female mortality, reproductive state of the females, number of eggs oviposited, and schedule changes in the photoperiod and temperature regimes which were instituted for each of the seven treatments.

Table XXII presents the treatment design that was followed for each of the treatments. Special note should be taken of the differences between the treatments with regard to the scheduling of the photoperiod and temperature regimes, the duration in days of these schedules, and the light intensity.

Table XXIII shows the number of males and females in each of two replicates for each treatment. This is shown in the column denoted as base total. It was not possible to maintain an equal sex ratio for each treatment for two reasons: 1) the overall sex ratio of males to females was 52.4 to 47.6, and 2) 46 males and 55 females in all were randomly removed from most of the treatments on July 22, 1971 for use in another experiment, thus disrupting the existing semblance of a uniform sex ratio among the treatments prior to the 22nd of July.

In this experiment three different types of controlled temperature cabinets were used. Two were used particularly in the earlier phases of the experiment while the third was used throughout the duration of the experiment (Table XXII). Treatments 2a and 2b were initially placed in a Forma model 13 growth chamber illuminated by six 14 watt cool white flourescent tubes. Treatments 3a, 3b, and 4a were initially placed in a 55.5 cm^3 constant temperature growth chamber illuminated by one 15 watt cool white flourescent tube. Treatments 1a and 1b were permanently placed in the Model E-7-H Environmental chamber as were the other treatments in the later phases of the experiment. Initially these treatments were illuminated by eight

TABLE XXII. Treatment design for the aestival diapause termination experiment showing treatment number, cage number, date when prescribed photoperiod and temperature regimes were initiated, the number of days under each regime and the light intensity of the photoperiod regime.

Treatment	Cage #	Date 1971	Photo-period (hr.)	Temperature Regime (hr.)	(°F)	Number of days Under Regime to 11-8-71	Light Intensity(fc)[1]
1a	5,10	6-17	16-8	12-12	80-60	25	1550c
		7-12	14-10	14-10	70-50	90	575c
		10-10	8-16	8-16	70-50	29	575c
1b	7,13	6-17	16-8	12-12	80-60	25	1550c
		7-12	14-10	14-10	70-50	18	575c
		7-30	12-12	12-12	70-50	21	575c
		8-20	10-14	10-14	70-50	27	575c
		9-16	8-16	8-16	70-50	53	575c
2a	2,14	6-17	14-10	14-10	70-50	43	105a
		7-30	12-12	12-12	70-50	21	575c
		8-20	10-14	10-14	70-50	27	575c
		9-16	8-16	8-16	70-50	53	575c
2b	4,12	6-17	14-10	14-10	70-50	115	105a
		10-10	8-16	8-16	70-50	29	575c

[1] Light intensity as measured outside the base of the cage.
a - Forma Model 13 Growth Chamber
b - 55.5 cm^3 growth chamber
c - Model E-7-H Environmental Chamber

TABLE XXII (Continued). Treatment design for the aestival diapause termination experiment showing treatment number, cage number, date when prescribed photoperiod and temperature regime were initiated, the number of days under each regime and the light intensity of the photoperiod regime.

Treatment	Cage #	Date 1971	Photo-period (hr.)	Temperature Regime (hr.)	(°F)	Number of days Under Regime to 11-8-71	Light Intensity(fc) [1]
3a	1,15	6-17	16-8	24	70	43	44b
		7-30	12-12	12-12	70-50	21	575c
		8-20	10-14	10-14	70-50	27	575c
		9-16	8-16	8-16	70-50	53	575c
3b	8,11	6-17	16-8	24	70	43	44b
		7-30	12-12	12-12	70-50	21	575c
		8-20	10-14	10-14	70-50	27	575c
		9-16	8-16	8-16	70-50	53	575c
4a •	9,16	6-17	8-16	24	70	43	55b
		7-30	12-12	12-12	70-50	21	575c
		8-20	10-14	10-14	70-50	27	575c
		9-16	8-16	8-16	70-50	53	575c

[1] Light intensity as measured outside the base of the cage.
a - Forma Model 13 Growth Chamber
b - 55.5 cm^3 growth chamber
c - Model E-7-H Environmental Chamber

TABLE XXIII. Diapause termination data indicating base total, mortality, and number of days to diapause termination based on two replicates for each treatment.

Treatment	Base Totals		Mortality to day of Diapause Termination or end of test		%Mortality Based on Base Total Within Replicate		%Mortality Based on Total Mortality for All Treatments		Base Total
	Male	Female	Males	Females	Males	Females	Male	Female	Males & Females
1a									
Sum	50	31	49	31					81
Mean					98.00	100	12.56	8.61	
1b									
Sum	38	44	13	28					82
Mean					34.21	63.63	3.34	7.78	
2a									
Sum	45	29	13	5					74
Mean					28.88	17.24	3.34	1.39	
2b									
Sum	42	42	38	40					84
Mean					90.48	95.42	9.74	11.11	
3a									
Sum	49	37	49	37					86
Mean					100	100	12.56	10.28	
Columns (1) (2)	(3)		(4)		(5)		(6)		(7)

TABLE XXIII (Continued). Diapause termination data indicating base total, mortality, and number of days to diapause termination based on two replicates for each treatment.

Treatment	Base Totals Male	Base Totals Female	Mortality to day of Diapause Termination or end of test Males	Mortality to day of Diapause Termination or end of test Females	%Mortality Based on Base Total Within Replicate Males	%Mortality Based on Base Total Within Replicate Females	%Mortality Based on Total Mortality for All Treatments Male	%Mortality Based on Total Mortality for All Treatments Female	Base Total Males & Females
3b									
Sum	37	30	17	20					67
Mean					45.95	66.67	4.36	5.56	
4a									
Sum	27	41	16	19					68
Mean					59.26	46.34	4.10	5.28	
TOTAL	278	253	195	180					542
Overall Mean					65.25	69.90	7.14	7.14	
Columns (1) (2)		(3)		(4)		(5)		(6)	(7)

TABLE XXIII (Continued). Diapause termination data indicating base total, mortality, and number of days to diapause termination based on two replicates for each treatment.

Treatment	Mortality Male & Female	%Mortality based on Base Totals Male & Female	%Mortality based on total for all treatments Male & Female	# of Days to Terminate Diapause
1a				
Sum	80			144(did not terminate)
Mean		98.76	10.66	
1b				
Sum	41			103
Mean		50.00	5.46	
2a				
Sum	18			105
Mean		24.32	2.40	
2b				
Sum	78			171(did not terminate)
Mean		92.74	10.40	
3a				
Sum	86			Did not terminate
Mean		100	11.46	
3b				
Sum	37			151
Mean		55.22	4.94	
Columns (1) (2)	(8)	(9)	(10)	(11)

TABLE XXIII (Continued). Diapause termination data indicating base total, mortality, and number of days to diapause termination based on two replicates for each treatment.

Treatment	Mortality Male & Female	%Mortality based on Base Totals Male & Female	%Mortality based on total for all treatments Male & Female	# of Days to Terminate Diapause
4a				
Sum	35			79
Mean		51.47	4.67	
Overall Mean	375	67.50	7.14	
Columns (1) (2)	(8)	(9)	(10)	(11)

48 inch, 110 watt flourescent tubes and two 60 watt incandescent bulbs, but this was later halved on July 12, 1971. Light intensity for each environmental chamber is given in Table XXII.

On November 8, 1971 those treatments and replicates which had terminated diapause were selected for a feeding and oviposition study. For these chosen replicates, data records were ceased after the eighth of November, but for the remaining treatments and replicates records were kept until all adults had died.

To further define which of the four treatments (1b, 2a, 3b, 4a) is the more efficient, a technique was devised (Table XXVI) which will be referred to as the diapause terminating efficiency factor (DTEF). The technique encompasses the following parameters: mean number of eggs per female adjusted for female mortality (column 4); number of days from day one of diapause termination to day x for which egg data is available, where x ranges from 13 to 35 days (column 2); mean number of eggs per female per day (column 5); mean number of days from June 17, 1971 required to terminate diapause (column 6); mean percent adult mortality based on total mortality for both sexes combined (column 7), for females (column 8) and for males (column 9); day-mortality factor (DMF) (columns 10-12) for combined sexes, females and males respectively; and finally, DTEF for combined sexes, females and males (columns 13-15, respectively).

The diapause termination efficiency factor takes into account each of the six parameters mentioned above and is calculated as follows:

the number of eggs per day is determined by dividing figures in column 1 by those in column 2 for the respective treatments. The number of eggs per female is adjusted for female mortality occurring during the oviposition period. This is determined by calculating the number of eggs per living female from the raw data throughout the duration of the oviposition period. The figures are summed and a mean adjusted figure for the number of eggs per female determined for each replicate. By summing the replicate figures and dividing by 2 an adjusted mean figure for the number of eggs per female is determined for each treatment. The number of eggs per female per day is calculated by dividing the replicate figures for the number of eggs per female (column 4) by the duration in days of the measured oviposition period (column 2). The number of days to terminate diapause for each replicate and the mean per treatment is given in column 6. The mean percent mortality for both males and females together and separately based on total mortality is shown in columns 7-9 as taken from columns 10 and 6 of Table XXIII. The day mortality factor as a means of expressing the mortality with regard to the number of days required to terminate diapause per treatment for both sexes together and separately are shown in columns 10, 11, and 12. It is calculated by determining the product of figures in column 6 and the appropriate figures from each column, 7, 8, or 9. Finally, the diapause termination efficiency factor for both sexes together or separately is determined by dividing the appropriate figure in columns 10, 11, or 12 by the mean number of eggs per female per day

(column 5). Greatest efficiency is demonstrated by the highest DTEF figure. Since six parameters are used in calculating this factor, I feel that it is a true indicator of the relative efficiency of each treatment with respect to photoperiod and temperature factors for the termination of aestival diapause in C. horridus.

RESULTS

Of the seven treatment means (Table XXIII), treatments 2a, 4a, 1b, and 3b are outstanding in having mean percent mortalities below 75 percent based on base totals within each replicate to the day of diapause termination or death of all weevils within each treatment. Treatments 2b, 1a, and 3a suffered percent mortalities above 92 percent. In addition, the females within treatment 3a and one replicate each in treatments 1a and 2b did not terminate diapause. Of the replicates in treatments 1a and 2b in which females terminated diapause, only 2 and 11 eggs were oviposited respectively. Thus, these three treatments, 1a, 2b, and 3a were disregarded from further analysis since they proved to be the least efficient means of terminating diapause with respect to mortality and number of days required for diapause termination.

Treatment 2a is consistently lowest in mean percent mortality for all three categories. Treatment 4b is next best, although it is third in percent male mortality. Treatment 3b is third, although it is fourth in percent male mortality. Finally, treatment 1b is fourth overall except for being second in percent male mortality.

An analysis of variance for percent mortality based on total mortality for all treatments to the day of diapause termination or death of all weevils within the treatment (Table XXIV) reveals that the percent mortality for each sex and for both sexes combined for the seven photoperiod-temperature regimes tested were significantly different at the .05 level. Duncan's new multiple range test carried out on the ranked treatment means for percent mortality based on total mortality to the day of diapause termination or death of all weevils within the treatment (Table XXV) gave the following number of significant differences among the ranked means for males, females, and both sexes respectively: .05 level - 8, 4, and 11; .01 level - 0, 0, and 2.

The results of the analytical technique can be viewed in several perspectives depending upon the desired objective. For overall DTEF for both sexes combined, treatment 2a with a value of 2059.70 is the best. Treatments 4a and 1b are second and third in efficiency having values of 1676.95 and 1301.81 respectively. Treatment 3b although lacking sufficient egg data for analysis can be ranked the least efficient in all categories by comparison of the known parameters of those of other treatments.

If female efficiency is the sole concern, then treatment 1b is most efficient with a value of 1854.95, followed by treatments 4a and 2a with values of 1896.00 and 1192.91 respectively. On the other hand, if male efficiency is of concern, treatment 2a is most efficient with a value of 2866.42 followed by treatments 4a and 1b

TABLE XXIV. F test of percent adult mortality based on total mortality for all treatments.

	F[a]	df[b]	$s_{\bar{x}}$[c]
Males	5.38*	7 & 6	1.86
Females	3.90*	7 & 6	1.69
Both sexes	5.87*	7 & 6	1.49

a = F test

b = degrees of freedom

c = standard error of mean

TABLE XXV. Mean percent adult mortality based on total mortality for treatments 1a, 1b, 2a, 2b, 3a, 3b, and 4a ranked according to Duncan's new multiple range test.

Treatments	Treatment Means	.05 [1/]	.01 [1/]
Males			
3a	12.56	a	a
1a	12.56	a	a
2b	9.74	ab	a
3b	4.36	b	a
4a	4.10	b	a
2a	3.34	b	a
1b	3.34	b	a
Females			
2b	11.11	a	a
3a	10.28	a	a
1a	8.61	a	a
1b	7.78	a	a
3b	5.56	ab	a
4a	5.28	ab	a
2a	1.39	b	a
Both sexes			
3a	11.46	a	a
1a	10.66	a	a
2b	10.40	ab	ab
1b	5.46	bc	ab
3b	4.94	c	ab
4a	4.67	c	ab
2a	2.40	c	b

[1/] Means in a given row with similar letters are not significantly different according to Duncan's new multiple range test.

TABLE XXVI. Technique for calculating the day-mortality factor and the diapause termination efficiency factor.

Treatment	Rep	(1) # of Eggs Oviposited During Observed Oviposition	(2) Duration of Observed Oviposition Pd.	(3) # of Eggs Per Day	(4) Adjusted # Eggs per Female for Mortality	(5) # Eggs Per Female Per Day	(6) # Days to Terminate Diapause
1b	1	54	22	2.45	2.42	.110	95
	2	222	13	17.08	9.80	.754	111
	Mean			9.76	6.11	.432	103
2a	1	30	35	0.86	2.22	.063	110
	2	187	24	7.79	4.92	.205	120
	Mean			4.32	3.57	.134	115
3b	1	11	-	-	0.69	-	144
	2	12	-	-	12.00	-	158
	Mean				6.34		151
4a	1	182	26	7.00	5.18	.199	80
	2	237	24	9.88	5.77	.240	78
	Mean			8.44	5.48	.220	79

TABLE XXVI (Continued). Technique for calculating the day-mortality factor and the diapause termination efficiency factor.

Treatment	Rep	(7) % Mortality Based on Total mortality (M&F)	(8) % Mortality Based on Total mortality (F)	(9) % Mortality Based on Total mortality (M)	(10) Day Mortality Factor (M&F)	(11) Day Mortality Factor Females	(12) Day Mortality Factor Males
1b	Mean	5.46	7.78	3.34	562.38	801.34	344.02
2a	Mean	2.40	1.39	3.34	276.00	159.85	38[illegible].10
3b	Mean	4.94	5.56	4.36	745.95	839.56	658.36
4a	Mean	4.67	5.28	4.10	368.93	417.12	323.90

TABLE XXVI (Continued). Technique for calculating the day-mortality factor and the diapause termination efficiency factor.

Treatment	Rep	(13) Diapause Termination Efficiency Factor (DTEF) Males & Females	(14) Females	(15) Males
1b	Mean	1301.81	1854.95	796.30
2a	Mean	2059.70	1192.91	2866.42
3b	Mean	-	-	-
4a	Mean	1676.95	1896.00	1472.27

with values of 1472.27 and 796.30 respectively.

If time is of major concern and the early termination of diapause the desired objective, then the treatment 4a photoperiod and temperature regime is the best with diapause termination occurring an average of 79 days after the beginning of the experiment. With this treatment, 50 percent adult mortality may be expected. If time is of less importance to the investigator and mortality more crucial, treatment 2a is probably more desirable and 25 percent mortality should be expected. On the other hand, if time is of some importance and many eggs oviposited per female per day also of importance, and mortality less so, treatment 1b is desirable with a probable mortality of 50 percent. Thus, three treatments (1b, 2a, and 4a) stand out as being the most efficient among all seven in terms of efficiency in terminating diapause.

DISCUSSION

Since all treatments except 4a were basically similar in design and the major differences among them were the duration of particular regimes or the initial photoperiod and temperature regimes applied to certain treatments, the results may only reflect these differences. The conservative nature of the treatments was designed to keep adult mortality to a minimum since the number of non-diapausing adults resulting from this study were critical to other planned experiments. Thus, to accurately depict photoperiod and temperature factors in relation to terminating diapause, a more liberal treatment design is necessary where mortality is of little concern. For these reasons,

the results of this experiment can only be viewed as preliminary and hopefully are reflective of trends which would be manifest in an experiment with a suitable treatment design.

The treatment selected for future use will depend upon the desired objective of the investigator. For example, if egg production is to be extended over a long period - up to three to five months so that larvae can be used for testing purposes for this period of time - all three treatment methods of diapause termination may be employed as was achieved by this investigator. A laboratory strain of bivoltine or possibly trivoltine weevils for the purpose of rapid mass production may be obtained by the application of short-day photoperiods of less than 10 hours for the first couple of generations. Rephasing the bi- or trivoltine cycle to a univoltine cycle can be executed by exposing teneral adults of a later generation to long photoperiods and warm temperatures to induce aestivation as it would normally occur in the summer and then diapause termination of the laboratory strain would be in phase with the natural life history of the insect. Frick and Johnson (1972) were able to change the normally univoltine cycle of the flea beetle Longitarsus jacobaeae to a bivoltine cycle in the laboratory by applying a 12 hour photophase at 24°C and a 12 hour scotophase at 12.75°C to all stages of the insect. Furthermore, Ankersmit (1960, 1964, 1965) avoided aestivation in the weevil Ceutorhynchus pleurostigma by exposing the teneral adults to short-day photoperiods and cool temperatures while aestivation was induced by long day photoperiods and warm temperatures

above 21°C. The key to the application of these techniques is to intentionally select for nondiapausing insects by consistently selecting progeny of early hatching eggs (Frick and Johnson, 1972, and Huggans and Blickenstaff, 1964). This type of selection unfortunately becomes difficult to reverse since the gene pool has been so diminished as to lack the depth or elasticity necessary to initiate the reverse selection process for diapausing insects. This problem was recently discussed by Frick and Johnson (1972) regarding the L. jacobaeae Swiss biotype. Selection for those eggs that had the shortest diapause over a 4 year period (8 generations) reduced the average length of diapause for the eggs from 17 to 6 weeks. Subsequently, the rearing of the Swiss biotype colony was terminated since these authors felt that this population probably would not survive in the field because of its artificially shortened duration of egg diapause which could cause the life cycle to be out of synchronization with the season in Pacific Coast states. Thus, by not consistently quasi-splitting gene pools via the selection process, and instead rearing the population as a whole self-contained unit, I feel that by the proper manipulation of the knowledge already known on the photoperiod and temperature effects, that it should theoretically be possible to mass rear C. horridus in not only great numbers but also readily adapted to the geographical locale in which one may wish to introduce it. The next stage in this investigation should be to develop a rearing media in which these endophytic larvae can survive and develop and to determine

whether the photoperiodic and temperature effects experienced by the larvae become manifest in the adults.

With regard to light intensity no definite statement can be made as to its effects in this experiment due to the presence of a number of other variables which confound any effects that may exist. Lees (1955) stated that generally the photoperiodic reaction in arthropods is independent of light intensity and total light energy provided the intensity exceeds the threshold; perception of white light is usually in the region of 1 foot-candle with dimmer illumination being equivalent to darkness.

Since only three treatments (3a, 3b, and 4a) were initially under a constant temperature regime and this was not maintained for the total duration of the experiment, the effects of this factor cannot be determined. Beck (1968) indicated that when the temperature is constant, the critical day length displayed by populations of experimental insects may vary according to the temperature employed. This may be manifest as a considerable modification or abolishment of the insect's reaction to photoperiod. The exact effect with regard to critical day length and the occurrence of diapause depends on the insect's individual response threshold, which is a genetic characteristic (Beck, 1968), and , in turn, is finely tuned by the geographical locale of the insect's habitat. Generally, at relatively low rearing temperatures, diapause may be induced at all photoperiods while no photoperiodic induction of diapause may occur under relatively high temperature conditions (Beck, 1968). This apparently applies for the long-day response while the

converse is more likely for the short-day response.

Thermoperiod (Bunning, 1964; Wilkins, 1965; and Beck, 1968) play a less decisive role than does photoperiod in phase-setting rhythmic functions with regard to circadian rhythms. Beck further stated that under some circumstances it will substitute for photoperiod in the determination of diapause and also modify the insect's response to photoperiod. Its effect depends upon the insect's reliance upon the naturally occurring thermoperiod for circadian entrainment as a regulator of its daily behavioral patterns.

SUMMARY AND CONCLUSIONS

From a study on aestival diapause termination for the teneral C. horridus adults, an efficient means of terminating diapause by manipulation of the photoperiod and temperature regimes was determined. Efficiency was determined in relation to the lowest percent mortality, the fewest number of days for diapause termination, and the earliest maximum period of egg production. Results of the experiment may be summarized as follows:

1. An extended long-day photoperiod (treatments 1a and 2b) tends to sustain the aestival diapause state and in each case results in high adult mortality and no termination of diapause.

2. A gradually reduced photoperiod (treatments 1b, 2a, and 3b) which coincides with the seasonal life history of C. horridus results in moderately low mortality and diapause termination ranging a mean of 103 to 151 days.

3. Teneral adults exposed to a short-day photoperiod (treatment 4a) resulted in moderately low mortality and diapause termination requiring a mean of 79 days.

4. For some unknown reason, treatments 3a and 3b, identical in every respect, resulted in drastic differences in which the former sustained high mortality and did not terminate diapause, while the latter sustained moderately low mortality and terminated diapause a mean of 151 days from the initiation of the experiment.

5. The diapause termination efficiency factor (DTEF), as defined in this study, indicates that for combined sexes efficiencies ranked in ascending order are treatments 3b, 1b, 4a, and 2a. The gradually reduced photoperiod (treatment 2a) is the most efficient, followed by the short-day photoperiod (treatment 4a). The choice of photoperiod and temperature regime (1b, 4a, or 2a) for future use will depend upon the desired objective of the investigator.

6. Based on these results, I feel that it is theoretically possible to mass rear C. horridus as a bivoltine and possibly trivoltine life cycle in the laboratory. Also, by proper manipulation of the photoperiod and temperature regime, I feel that it is possible to mass rear this weevil so that it is readily adapted by means of artificially induced selection to practically any geographical locale to which one may wish to introduce it.

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APPENDIX A

C. horridus Bibliography

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APPENDIX B

Raw Data for Tables IX and X

TABLE I. Raw data for Table IX giving the results of the primary host specificity test for the entire experiment. Rep I - Group I

	# Days to Death of last instar	# Reaching 2nd instar	# Reaching 3rd instar	Tunnel Length (mm)
C. nutans	10-*	0	0	33.72
C. acanthoides	10-	0	0	13.82
C. pycnocephalus	10-	0	0	26.08
C. horridissimus	10-	0	0	36.18
S. marianum	10-	0	0	40.69
C. arvense	10-	0	0	23.72
C. vulgare	115-*	2	2	225.39
C. acaulis	25-	0	0	16.57
O. acanthium	26-	2	0	123.43
X. annum	30-	1	0	133.63
C. montana	5-	0	0	21.37
C. nigrescens	50-	1	0	70.20
C. cyanus	5-	0	0	1.47
		Rep II		
C. nutans	10-	0	0	29.12
C. acanthoides	55-	3	3	232.16
C. pycnocephalus	5-	0	0	15-29
C. horridissimus	5-	0	0	21.67
S. marianum	10-	0	0	24.61
C. arvense	81-	3	2	202.45
C. vulgare	10-	0	0	31.37
C. acaulis	25-	0	0	7.16
O. acanthium	10-	0	0	33.72
X. annum	35-	0	0	114.12
C. montana	10-	0	0	16.96
C. nigrescens	15-	0	0	16.80
C. cyanus	10-	0	0	4.41
		Rep III		
C. nutans	116-	3	3	262.06
C. acanthoides	80-	4	3	218-14
C. pycnocephalus	5-	0	0	28.14
C. horridissimus	25-	0	0	37.74
S. marianum	5-	0	0	11.57
C. arvense	89-	2	1	114.02
C. vulgare	15-	1	0	64.12
C. acaulis	9-	0	0	9.61
O. acanthium	75-	2	2	367.65
X. annum	29-	1	0	110.49
C. montana	15-	0	0	30.59
C. nigrescens	30-	0	0	53.14
C. cyanus	20-	0	0	38.72

TABLE I (Continued) - Group II

	# Days to Death of last instar	# Reaching 2nd instar	# Reaching 3rd instar	Tunnel Length (mm)
Rep I				
C. nutans	10-	0	0	13.24
C. scolymus	5-	0	0	5.39
C. cardunculus	5-	0	0	27.45
H. annus	5-	0	0	19.32
L. sativa	20+	2	0	124.41
C. sultana	10-	0	0	11.47
Rep II				
C. nutans	5-*	0(1)	0(1)	33.14
C. Scolymus	5-	0	0	8.24
C. cardunculus	5-	0	0	12.84
H. annus	5-	0	0	9.90
L. sativa	109+*	1	1	377.74*
C. sultana	20-	0	0	41.67
Rep III				
C. nutans	5-	0	0	17.94
C. scolymus	5-	0	0	36.37
C. cardunculus	5-	0	0	12.74
H. annus	5-	0	0	17.45
L. sativa	17-	2	0	153.82
C. sultana	5-	0	0	6.96

TABLE I (Continued). Group III

Rep I

	#Days to Death of last instar	# Reaching 2nd instar	# Reaching 3rd instar	Tunnel Length(mm)
C. nutans	5-*	0(1)	0(1)	22.84
Chrysanthemum sp.	10-	0	0	8.72
S. arenarius	10-	0	0	9.51
Aster sp.	5-	0	0	5.29
Inula sp.	5-	0	0	38.92
C. intybus	10-	0	0	14.41
C. endiva	5-	0	0	7.06
T. erecta	15-	0	0	14.71
A. vulgaris	10-	0	0	4.80
Helichrysum sp.	10-	0	0	3.14
T. officinale	5-	0	0	10.69
	Rep II			
C. nutans	5-*	1	1	31.96
Chrysanthemum sp.	10-	0	0	9.12
S. arenarius	15-	0	0	22.16
Aster sp.	10-	0	0	5.39
Inula sp.	10-	0	0	19.22
C. intybus	10-	0	0	3.53
C. endiva	5-	0	0	0.00
T. erecta	10-	0	0	5.39
A. vulgaris	10-	0	0	4.31
Helichrysum sp.	15-	0	0	59.61
T. officinale	10-	0	0	14.41
	Rep III			
C. nutans	5-*	0	0	30.69
Chrysanthemum sp.	10-	0	0	5.98
S. arenarius	10-	0	0	21.57
Aster sp.	5-	0	0	5.39
Inula sp.	10-	0	0	14.41
C. intybus	5-	0	0	5.49
C. endiva	5-	0	0	7.35
T. erecta	10-	0	0	10.20
A. vulgaris	5-	0	0	4.90
Helichrysum sp.	10-	0	0	19.61
T. officinale	10-	0	0	19.12

TABLE I (Continued) -Group IV

	#Days to Death of last instar	# Reaching 2nd instar	# Reaching 3rd instar	Tunnel Length (mm)
	Rep I			
C. nutans	20+	2	1	70.39
C. frutescens	10-	0	0	2.84
L. esculentum	10-	0	0	7.45
R. sativa	15-	0	0	17.06
B. vulgaris	10-	0	0	3.63
D. carota	5-	0	0	8.53
B.o. botrytis	25-	0	0	25.20
Z. mays	5-	0	0	0.98
M. sativa	5-	0	0	20.29
	Rep II			
C. nutans	20-*	3	1	69.02
C. frutescens	10-	0	0	7.65
L. esculentum	10-	0	0	16.76
R. sativa	15-	0	0	12.94
B. vulgaris	10-	0	0	9.12
D. carota	10-	0	0	13.82
B.o. botrytis	20-	0	0	13.14
Z. mays	10-	0	0	12.94
M. sativa	10-	0	0	28.82
	Rep III			
C. nutans	15-*	0	0	51.96
C. frutescens	5-	0	0	4.51
L. esculentum	10-	0	0	13.04
R. sativa	15-	0	0	14.12
B. vulgaris	10-	0	0	8.24
D. carota	10-	0	0	16.67
B.o. botrytis	25-	0	0	45.88
Z. mays	5-	0	0	1.67
M. sativa	10-	0	0	50.69

( ) plant dissected and larva found.

* plant infested with 1 C. horridus larva from subsequent reinfestation.

TABLE II. Raw data for Table X giving the results of the primary host specificity test for the first five days of the experiment. Group I

Rep I

	# Dead- 5 days	# cf - 5 days	# Alive 5 days	Body Length Growth(+)-(-)	Tunnel Length (mm)
C. nutans	0	0	5	5+	18.82
C. acanthoides	3	1	1	4-	13.14
C. pycnocephalus	0	4	1	1nc	17.06
C. horridissimus	0	3	2	1+ 1-	30.20
S. marianum	0	4	1	1+	21.57
C. arvense	1	2	2	1+ 1-	23.43
C. vulgare	0	1	4	3+ 1nc	14.51
C. acaulis	4	0	1	1+	14.41
O. acanthium	1	2	2	2+ 1-	27.35
X. annum	3	0	2	2+ 3-	17.65
C. montana	4	1	0	4-	21.37
C. nigrescens	1	0	4	3+ 2-**	20.78
C. cyanus	4	1	0	4-	1.47

Rep II

C. nutans	0	4	1	1+	24.80
C. acanthoides	0	2	3	3+	40.98
C. pycnocephalus	2	3	0	2-	15.29
C. horridissimus	0	5	0	--	20.69
S. marianum	0	4	1	1-	20.49
C. arvense	1	1(1)	3(1)	3+ 1-	40.09
C. vulgare	3	1	1	4-	31.37
C. acaulis	3	0	2	5-	3.33
O. acanthium	1	1	3	2+ 1nc 1-	16.47
X. annum	1	1	3	3+ 1-	17.45
C. montana	4	0	1	5-	14.41
C. nigrescens	2	0	2	4-	7.45
C. cyanus	1	1	3	4-	1.96

Rep III

C. nutans	0	0	5	5+	43.23
C. acanthoides	2	0	3	3+ 2-	35.20
C. pycnocephalus	0	5	0	--	28.14
C. horridissimus	1	1	3	3-	19.61
S. marianum	2	3	0	2-	11.57
C. arvense	0	3-(1)	2+(1)	2+	47.06
C. vulgare	0	3	2	2+	40.00
C. acaulis	3	0	2	5-	8.14
O. acanthium	0	2	3	2+ 1-*	31.86
X. annum	2	0	3	5-	27.65
C. montana	2	0	3	1+ 1nc 3-	13.53
C. nigrescens	1	1	3	3+ 1-	17.06
C. cyanus	1+ 1ac	0	3	2+ 1nc 2-	14.90

TABLE II - Continued - Group II

	# Dead-5 Days	# [illegible] 5 Days	# Alive 5 Days	Body Length Growth (+)-(-)	Tunnel Length (mm)
Rep I					
C. nutans	3	0	2	2nc 3-	12.25
C. scolymus	3	1	1	4-	5.39
C. cardunculus	2	3	0	2-	27.45
H. annus	3	2	0	1nc 2-	19.31
L. sativa	2	0	3	3+ 2-	62.35
C. sultana	1	1	3	3-	7.94
Rep II					
C. nutans	0	5	0	--	33.14
C. scolymus	3	2	0	3-	8.23
C. cardunculus	3	2	0	3-	12.84
H. annus	4	1	0	4-	9.90
L. sativa	2	1	2	2+ 2-	41.67
C. sultana	0	0	5	3+ 2-	15.49
Rep III					
C. nutans	0	4	1	1-	17.94
C. scolymus	1	4	0	1-	36.67
C. cardunculus	5	0	0	5-	12.74
H. annus	5	0	0	5-	17.45
L. sativa	0	2	3	3+	87.94
C. sultana	5	0	0	5-	6.96

TABLE II (Continued) - Group III

Rep I

	# Dead- 5 Days	# cf- 5 Days	# Alive 5 Days	Body Length Growth (+)-(-)	Tunnel Length(mm)
C. nutans	0	5(1)	0(1)	1+	22.84
Chrysanthemum sp.	2	0	3	2+ 3-	8.63
S. arvense	3	1	1	1-	7.55
Aster sp.	5	0	0	5-	5.29
Inula sp.	4	1	0	4-	38.92
C. intybus	2	0	3	2+ 3-	12.06
C. endiva	3	2	0	3-	7.06
T. erecta	4	0	1	5-	4.02
A. vulgaris	4	0	1	1+ 4-	4.12
Helichrysum sp.	3	0	2	5-	1.96
T. officinale	4	1	0	4-	10.69

Rep II

C. nutans	0	5	0	--	31.96
Chrysanthemum sp.	4	0	1	5-	8.72
S. arvense	1	0	4	2+ 2-	15.49
Aster sp.	4	0	1	1+ 4-	5.39
Inula sp.	1	0	4	2+ 3-	4.90
C. intybus	3	0	2	5-	3.53
C. endiva	1	4	0	--	0.00
T. erecta	2	2	1	3-	5.69
A. vulgaris	4	0	1	5-	4.31
Helichrysum sp.	2	1	2	2+ 2-	35.20
T. officinale	2	0	3	4-	14.41

Rep III

C. nutans	2	3	0	2-	30.69
Chrysanthemum sp.	2	0	3	1+ 4-	3.72
S. arvense	2	0	3	3+ 2-	16.76
Aster sp.	4	1	0	4-	5.39
Inula sp.	1	0	4	1+ 4-	10.78
C. intybus	5	0	0	5-	5.49
C. endiva	5	0	0	5-	7.35
T. erecta	3	0	2	1+ 3-	8.53
A. vulgaris	4	1	0	5-	4.90
Helichrysum sp.	0	0	5	5-	14.22
T. officinale	1	0	4	1+ 4-	17.84

TABLE II (Continued) - Group IV

Rep I

	# Dead 5 Days	# cf 5 Days	# Alive 5 Days	Body Length Growth (+)-(-)	Tunnel Length
C. nutans	0	0	5	3+ 2-	19.31
C. frutescens	2	1	2	4-	1.67
L. esculentum	1	0	4	1nc 4-	4.51
R. sativa	0	1	4	1+ 3-	4.80
B. vulgaris	4	0	1	5-	2.16
D. carota	4	1	0	4-	8.72
B.o. botrytis	0	0	5	4+ 1nc	7.45
Z. mays	5	0	0	5-	0.98
M. sativa	5	0	0	1+ 4-	20.29
		Rep II			
C. nutans	1	2	2	2+1-	24.12
C. frutescens	4	0	1	1+ 4-	6.96
L. esculentum	0	0	5	5-	7.25
R. sativa	0	1	4	1+ 3-	6.67
B. vulgaris	3	0	2	3-	7.84
D. carota	0	0	5	1+ 4-	8.82
B.o. botrytis	1	1	3	1+ 2nc	7.45
Z. mays	3	0	2	2+ 3-	12.94
M. sativa	3	1	1	1nc 3-	24.02
		Rep III			
C. nutans	1	3	1	1+	28.14
C. frutescens	5	0	0	5-	4.51
L. esculentum	2	0	3	5-	10.00
R. sativa	0	4	1	1-	12.55
B. vulgaris	2	1	2	4-	6.27
D. carota	0	0	5	1+ 2nc 2-	9.02
B.o. botrytis	1	1	3	2+ 1nc 1-	15.49
Z. mays	5	0	0	5-	1.67
M. sativa	4	0	1	5-	48.33

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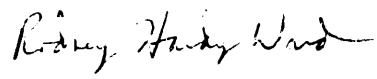
* actually 3+, total 7+

** actually 4+ 1-, total 7+

VITA

Rodney Hardy Ward was born January 27, 1946, in Keene, New Hampshire. In June of 1964 he was graduated from Wheatland-Chili Central School in Scottsville, New York. The following September he enrolled in the College of Agriculture at Cornell University, Ithaca, New York. As an undergraduate at Cornell he was employed as a research assistant for the Department of Entomology. In June of 1969 he was awarded the B.S. in Entomology from Cornell.

In September of 1970, Mr. Ward was enrolled as a graduate student in the Department of Entomology at Virginia Polytechnic Institute and State University. Presently, he is June candidate for the Master of Science degree in Entomology.



CEUTHORRHYNCHIDIUS HORRIDUS PANZER (COLEOPTERA: CURCULIONIDAE) - THE HOST SPECIFICITY OF THE FIRST INSTAR, NOTES ON THE INSECT'S BIOLOGY, AND METHODS OF ADULT AESTIVAL DIAPAUSE TERMINATION

by

Rodney H. Ward

The subtribe Carduinae (Compositae: Cynareae) consists of many thistle species which are becoming an ever increasing economic menace to range and pasture lands. A phytophagous weevil, Ceuthorrhynchidius horridus Panzer has been found to be associated with these weeds in Europe, but cannot be introduced without a comprehensive investigation of its host specificity and biology.

First instar host specificity tests on 36 species of economically and aesthetically important plants revealed an approximate host range among some species based on development to only the third instar. Field host range information disclosed that this weevil has not been reported to be a serious pest of any economic plant species, although it was cited to have been found on some important agronomic species.

An adult aestival diapause termination experiment indicated that diapause was most efficiently terminated with a gradually reduced long - day photoperiod while it was achieved quickest with a short-day photoperiod.