SARE Project AS93-8

Transmission of *Puccinia carduorum* by the Musk Thistle Herbivores, *Cassida rubiginosa* (Coleoptera: Chrysomelidae), *Trichosirocalus horridus* and *Rhinocyllus conicus* (Coleoptera: Curculionidae)[1]

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J. Entomol. Sci. 29(2): 186 -191 (April 1994)

ABSTRACT An autoecious rust fungus, *Puccinia carduorum* Jacky (Uredinales), imported from Turkey has been introduced for the biological control of musk thistle in Virginia. Studies were conducted to determine if this rust can be transmitted by the three thistle herbivores, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae), *Trichosirocalus horridus* (Panzer), and *Rhinocyllus conicus* Froelich (Coleoptera: Curculionidae). Fifty adults of each species were introduced to diseased plants and subsequently transferred to healthy test plants. Fifty adults introduced on and transferred to rust-free plants served as control. Spore dispersal by adults of the three coleopteran species was confirmed. Under greenhouse conditions, pustules developed within two weeks on some test plants where spore-carrying insects were introduced. Most of the urediniospores were observed on setae of legs of all adults.

KEY WORDS *Puccinia carduorum,* rust fungus, *Cassida rubiginosa, Trichosirocalus horridus, Rhinocyllus conicus,* thistle insects, rust transmission.

Successful establishments of two weevils [*Rhinocyllus conicus* Froelich and *Trichosirocalus horridus* (Panzer)] for the biological control of musk thistle (*Carduus thoermeri* Weinmann) in the United States has been well documented (Surles et al. 1974, Rees 1977, Puttler et al. 1978, Kok and Trumble 1979, Trumble and Kok 1982, Kok 1986, Kok and Roberts 1987, McAvoy et al. 1987). More recently, *R. conicus* has become established in some southern states, e.g. Tennessee (Lambdin and Grant 1992) and Georgia (Buntin et al. 1993), where the musk thistle problem has become widespread. *R. conicus* attacks the thistle buds, while *T. horridus* feeds on the rosettes. A third herbivore, *Cassida rubiginosa* Müller, a chrysomelid accidentally introduced into North America, complements the impact of the two weevils, as it is a foliage feeder (Cartwright 1983). Since *C. rubiginosa* fills a niche different from that of *T. horridus* and *R. conicus,* it supports the multiple stress concept for weed control.

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The addition of a pathogenic stress factor was presented with *Puccinia carduorum* Jacky (Uredinales), a musk thistle-infecting autoecious rust obtained from Turkey (Politis et al. 1984, Politis and Bruckart 1986). This rust fungus is the first pathogen introduced as a biological control agent of musk thistle in North America. Field evaluation of this rust in Virginia showed that it overwintered successfully on musk thistle and contributes to the control of the weed by seed reduction (Baudoin et al. 1993). Surveys conducted in the vicinity of rust-infected plants indicated that rust pustules were found on wild thistle plants 100 to 200 m away. On these infected plants, insect damage, predominantely by *C. rubiginosa,* was always present. Although wind is obviously the most important agent in the spread of rust spores under natural conditions (Arthur et al. 1929), disease transmission by insects is well-known (Arthur et al. 1929, Strobel and Mathre 1970, Stevens 1977, Thresh et al. 1981, Lucas et al. 1985). Thus, the three thistle herbivores may disperse rust propagules. To investigate this possibility, studies on transmission of *P. carduorum* by the three musk thistle herbivores were conducted.

Materials and Methods

Acquisition of rust inoculum by herbivores from infected plants. Field plots of musk thistles at the Prices Fork Research Center, Montgomery Co. VA were inoculated with urediniospores of *P. carduorum* (isolate III from Turkey) produced on musk thistle in a greenhouse at the Foreign Disease and Weed Research Unit, USDA-ARS, Fort Detrick, Frederick, Maryland (Baudoin et al. 1993) in 1987 and 1988. This site was selected because it was isolated from other musk thistle infestations and was well established with insects released as thistle biological control agents. From May to June 1989, several hundred field-collected adult insects (*C. rubiginosa, T. horridus,* and *R. conicus*) were caged as described below on rust-infected plants. After 24 h, the appendages of the insects were examined for rust spores under a compound microscope (44X).

Transmission Experiment. A series of transmission tests was carried out from May through July, 1989. Adults of the three insect species were collected from the Whitethorne Research Farm in Montgomery Co., VA, more than 10 km from the rust-inoculated plots at the Prices Fork Research Center, and maintained in a laboratory until they were used for the experiments. Musk thistle plants grown in pots in the greenhouse were used as test plants at the 10- to 15-leaf rosette stage.

At least 50 adults of each of the three insect species were caged on a potted, healthy plant. The cylindrical cage was made of plastic with nylon mesh on one end and the other fitted over the potted plant. Another set of 50 or more insects of each species was introduced and caged in the same manner on a rust-infected plant. After 24 h, individuals of each insect species were picked up by forceps and transferred to respective potted healthy plants. Based on the expected amount of feeding by the three insect species, each plant was caged with 5 *C. rubiginosa,* 10 *T. horridus,* or 10 *R. conicus*. Five test plants per insect species in each group (rust and insect-control group) were used, each plant representing a replicate. Another set of five plants without insect introduction

served as an insect-free control. All insects, starting with the insect-control group, were removed from the test plants the following day. The plants were placed under a time-regulated fine water mist for at least 24 h, maintained at 25-30°C in the greenhouse, and monitored for development of disease symptoms. The number of infected plants and number of rust pustules observed per plant for each herbivore, and time for rust symptoms to appear were recorded. This experiment was repeated three times. Significant differences in infection of musk thistle by *P. carduorum* transmitted by the three herbivores were determined by Tukey's test.

Results and Discussion

Acquisition of rust inoculum by herbivores from infected plants. Rust urediniospores were consistently observed on each of the three insects. Although no counts were made, most spores were observed adhering to the setae on the legs of the beetles (Figure 1). All three insects consistently acquired the rust spores on their legs. Occasionally, spores were also found along the snout of *T. horridus*. This confirms that adult beetles feeding on rust-infected plants readily encounter the spores which are then carried with them when they move to another plant.

Transmission Experiment. With each insect species, rust pustules appeared on test plants within 2 weeks from inoculation. However, not all plants manifested rust infection. More than half the plants exposed to each insect species developed rust symptoms; the proportion of infected plants did not vary significantly with herbivore. Likewise, the number of pustules per plant per adult did not differ between insect species (Table 1). Plants in the two control groups (insect and insect-free) did not develop any pustules. This confirmed that the transmitted spores were vectored by beetles in contact with the rust infected plants.

The study demonstrated that *C. rubiginosa, T. horridus* and *R. conicus* can transport rust propagules from diseased to healthy plants. However, how long or how far they carry the spores remains to be investigated. The wide variation in the number of pustules per plant is indicative of the high variability in the amount of inoculum acquired and transmitted by the insects.

Transmission may be more efficient (at least for short distances) under field conditions, since there is continuous insect movement (for feeding or oviposition) from plant to plant. Because spores are also efficiently dispersed by wind over short distances, the more important role of the herbivores in spore dissemination may be in long distance transportation as the insects search for new hosts. The host searching and dispersal ability of *T. horridus* (McAvoy et al. 1987) and of *R. conicus* (Kok and Surles 1975) have been well documented. *C. rubiginosa,* which was accidentally introduced into Canada, has dispersed southwards to Virginia on its own (Cartwright 1983). Thus, if these three herbivores are able to transmit the rust spores, the spread of *P. carduorum* can be hastened and vectored by insects to other infestations of musk thistle. This would add to the natural wind dispersal of the rust.

Another way insects can help is dispersal of the propagules over short distances when the musk thistles are in the rosette stage and their leaves are

Fig. 1. Photomicrographs of legs of the 3 herbivores showing adhering urediniospores (indicated by arrows) of *Puccinia carduorum*: a - *Cassida rubiginosa,* b - *Trichosirocalus horridus,* and c - *Rhinocyllus conicus.*

Table 1. Infection of musk thistle by *Puccinia carduorum* using three biocontrol agents as inoculum carriers.

Category	Herbivore*		
	Rhinocyllus conicus	*Trichosirocalus horridus*	*Cassida rubiginosa*
Plants infected (%)	53.3 ± 11 ns	73.3 ± 14.4 ns	53.3 ± 19.6 ns
Pustules/plant/adult (No.)	1.3 ± 0.6 ns	1.2 ± 0.6 ns	2.0 ± 0.8 ns

*Mean of 3 repetitions (15 test plants per trial; 5 replicates/herbivore); analysis for % was based on arcsine transformation.
ns = not significantly different for each row ($p > 0.05$) HSD, Tukey's Test.

laying almost flat to the ground. In this case, spore dispersal by wind may be less efficient, as shown by consistently low levels of infection (Baudoin et al. 1993). First appearance of overwintered insects of all three species is detected when thistles are in the rosette stage in late winter or early spring. Increased rust infection at the rosette stage would weaken the thistle and decrease its ability to compete against other vegetation. Although the three species of beetles were found to carry large numbers (> 20 per leg) of rust spores in this study, none appeared to be adversely affected by the fungus. Determination of the influence of the rust on the feeding behavior and oviposition of the three herbivores merits further investigation.

The direct impact of the rust on seed production of musk thistle is less than that of each of the herbivores, but it contributes to seed reduction (Baudoin et al. 1993). Compatibility of the insects and the rust fungus, with a net increase in their overall effectiveness, lends credence to the multiple stress concept successfully used in the biological control of musk thistle (Trumble and Kok 1982).

Acknowledgments

We thank A. B. A. M. Baudoin, Department of Plant Pathology, Physiology and Weed Science, VPI&SU for assistance with inoculation of the rust. This project was funded in part by a cooperative grant from the Foreign Disease and Weed Research Unit, USDA-ARS, Fort Detrick, Frederick, MD.

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