Rearing the wheat stem sawfly on an artificial diet

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The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is an insect pest in dryland wheat cropping systems in the southern Canadian Prairies and the northern Great Plains of the United States (Morrill 1997). Yield losses caused by *C. cinctus* are due to reduced head weight (Holmes 1977; Morrill *et al.* 1992) and lodging, which decreases harvest efficiency. Estimates of yield losses in Montana alone are about US$25 million per year.

The biology of *C. cinctus* is well known. It has a wide host range including nearly all large-stemmed grasses except oats, *Avena sativa* L. (Farstad 1940). Adults emerge from fallow fields and migrate into crop fields as wheat stems elongate, from mid-May to late July (Morrill and Kushnak 1999). Eggs are laid in stems, and larvae feed on the parenchyma (Ainslie 1920) of growing wheat plants. Plants are susceptible from stem elongation through flowering. Mature larvae move to the base of the plant and cut notches around the inner perimeter of the stem near ground level, which usually causes lodging and loss of wheat heads at harvest (Ainslie 1920). The larvae overwinter in diapause within the lower section of the cut stem (Ainslie 1920). Diapause is completed after about 3 months at low temperature (Salt 1947), and pupation occurs in the spring.

New basic research studies would be possible if *C. cinctus* larvae could be reared on artificial media. Currently, larvae can be reared only in plant stems, which must be reared in a glasshouse or be collected from the field. Both topical and consumption dose-response studies are difficult to quantify with insects that are feeding within plant stems. Off-season adults can be obtained by refrigerating field-collected diapausing larvae. Pupation and adult eclosion will occur following incubation at room temperature. Adults readily mate in the laboratory, and artificial oviposition sites can be provided to obtain eggs.

The first reported attempt to rear *C. cinctus* larvae on artificial diet used natural foods such as wheat juice and cellulose powder (Kasting and McGinnis 1958). Although *C. cinctus* were reared on a diet mixture (Kasting and McGinnis 1958), larval survival was limited to 14 days, and it was necessary to provide fresh diet every 24–48 h (McGinnis and Kasting 1962). Identified problems included mold contamination and the configuration of the diet (McGinnis and Kasting 1962).

The legless *C. cinctus* larvae are adapted to feeding within an enclosed tube, or plant stem. To simulate these conditions, McGinnis and Kasting (1962) improved larval survival by placing evenly spaced plastic plates with grooves in the media. Since then, there have been no reports of improved methods for rearing *C. cinctus* larvae. Development of a satisfactory artificial medium, based on a better understanding of nutritional requirements and presentation of diet, would open new opportunities for our current research on larval antibiosis and fungal pathogens.

This paper presents a new diet medium for the wheat stem sawfly that was successfully used to rear neonate larvae to maturation.

In the first experiment, a commercial diet for fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), consisting of sucrose, cholesterol, methylparaben, sorbic acid, vitamin mix (Vanderzant), ascorbic acid, stabilized wheat germ, casein, sterile corn cob grits, Wesson salt mix, and raw linseed oil (diet F9179B, BioServ, Frenchtown, New Jersey), was tested. The dry mixture was combined with 1 L of boiling agar (A-7002, SIGMA Chemical Co., St. Louis, Missouri) and blended for 2 min. The final mixture was dispensed immediately into 60 mm × 15 mm sterilized disposable plastic petri dishes (Fisher brand, Fisher

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Scientific, Hampton, New Hampshire). Petri dishes containing diet medium were cooled either under a purified vertical clean bench (Labconco Corp., Kansas City, Missouri) with UV light or on the laboratory bench for 30 min. Diets were sealed under petri-dish lids and then stored at 4 °C in a chromatography refrigerator (Isotemp™, Fisher Scientific) until larvae were available.

Before placing larvae in the media, either grooves (35 mm × 4.34 mm × 4.34 mm) or holes (4.34 mm × 4.34 mm) were made in the diet medium using a stainless-steel microspatula (Daigger Lab Supplies, Vernon Hills, Illinois) according to the method used by McGinnis and Kasting (1962). Neonate larvae were collected from field-infested stems and placed singly within individual grooves or holes using a camel's-hair brush. Petri dishes were held in an environmental chamber (Conviron, Winnipeg, Manitoba) at 21 °C and 40%–50% RH under complete darkness. This temperature and RH duplicated the average of hourly temperatures and RH that we measured in a wheat canopy in a local field at Churchill, Montana, from 17 June to 20 August, using a HOBO® RH/temperature data logger (H8, Onset Computer Co., Pocasset, Massachusetts).

Survival and larval development were determined twice per week by counting the number of live larvae and noting the presence of tunnels, which indicate feeding. Larvae were transferred to new media throughout the study as needed because of desiccation or contamination with microorganisms.

A total of 113 newly hatched larvae were used in this experiment. Larval mortality was 75.2% after 10 days. There was no significant difference in mortality rates of larvae between grooves and holes. Most mortality (~20%) was associated with growth of mold on the diet medium. It seems that despite transfer of larvae to new media as needed because of mold development, we were not able to avoid the negative effect of mold on larval survivorship. The growth of microorganisms (unknown species) or the presence of any other lethal contaminant on the medium may have been related to infection by contaminated tools, such as the camel's-hair brush and the microspatula used to make the grooves and holes. In addition, because we used field-collected larvae, it is possible that fungi were transported from the field on larvae.

Larval desiccation was also a major mortality factor. On many occasions, larvae moved out of the groove or hole to the drier top of the diet medium. This up-and-down (geotropic) movement throughout the feeding source is often observed in natural conditions (Holmes 1954), but in this study it may have had an inhibitory effect on feeding, since the surface of the medium is less moist and may have been an unacceptable food source. Inhibition of feeding could be due not only to water loss from the surface of the medium but also to the need for enclosure provided by the grooves and holes. Even though we did not observe any significant difference in larval survival or tunneling between grooves and holes, the larvae tended to start feeding and tunneling sooner when placed inside holes. Transfer of larvae from one petri dish to another, which took place approximately every 13 days, also caused mortality through injury or contamination by the camel's-hair brush. We did not observe significant differences in survival between UV-treated and untreated media. Although larval development on the medium seemed slower than development of field-collected larvae (personal observation), a total of five larvae (4.4%) survived for more than 60 days (29 June – 2 September). Despite their healthy appearance, larvae surviving for more than 60 days stopped tunneling.

In the second experiment, two diets were tested: (1) a modified version of the commercial artificial diet for *S. frugiperda* described previously, containing 1.3% chlortetracycline HCl (Aureomycin®, Fort Dodge Animal Health, Overland Park, Kansas) and 1.04% bicyclohexylammonium fumagilin (Fumagilin-B, Medivet Pharmaceuticals Ltd., High River, Alberta); and (2) a diet developed for rearing of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), by Lewis and Lynch (1969), composed of agar (13.3%), wheat germ (24.7%), dextrose (18.9%), cholesterol (1.5%), vitamin-free casein (20.9%), salt No. 2 (6.8%), methylparaben (0.9%), L-ascorbic acid (5.7%), vitamin supplement (4.4%), sorbic acid (0.4%), chlortetracycline HCl (1.3%), and bicyclohexylammonium fumagilin (1.04%).

For the second diet, agar was mixed in 1 L of cold water and boiled for 9 min. Wheat germ was then added and cooked for about 15 min. Once the wheat–agar mixture was cooked, it was blended with the dry ingredients for 3 min. The final mixture was then poured into a kettle and mixed at high speed for an additional minute. Immediately after mixing, the final diet

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Table 1. Mortality (%, mean ± SEM) of *Cephus cinctus* larvae after 10 days.

Experiment	Diet	Parameter	Mortality after 10 days
1	1	Groove	34.6±3.1*a*
		Hole	40.6±4.9*a*
2	2	Petri dish	3.8±1.3*aB*
		Drinking straw	8.4±3.3*aB*
	3	Petri dish	30.6±2.3*aC*
		Drinking straw	36.1±3.2*aC*

Note: Diet 1, *Spodoptera frugiperda* commercial diet; diet 2, modified *S. frugiperda* diet; diet 3, *Ostrinia nubilalis* diet. Percentages followed by the same letter are not significantly different (α = 0.05). Lowercase letters represent comparisons among rows. Uppercase letters represent comparisons among columns ($P < 0.0001$).

medium was dispensed into two types of container: (1) 60 mm × 15 mm sterilized disposable plastic petri dishes (Fisher brand, Fisher Scientific) or (2) 10-cm transparent plastic drinking straws. Diets were cooled only at the laboratory bench, since we did not observe any significant effects of UV light treatment during the first experiment.

Grooves and holes were made on the diet medium before placement of the larvae, as previously described, using sterilized tools. Holes were made in the soft medium within the drinking straws by inserting a sterilized pipette tip and removing it after the medium had solidified. Neonate larvae from field-infested stems were placed singly inside either grooves or holes, using a sterilized camel's-hair brush. Containers were held in an environmental chamber (Conviron) at 21 °C and 40%–50% RH under complete darkness. Survival and larval development were recorded twice per week. Larvae were transferred to new diet medium as needed because of desiccation or contamination with microorganisms.

Data were analyzed using a χ^2 test to compare the observed and predicted mortality (α = 0.05).

A total of 32 newly hatched larvae were used to evaluate the modified *S. frugiperda* diet. Sixteen larvae were placed singly in sterilized plastic petri dishes within either grooves or holes and 16 were placed singly inside the plastic drinking straws. There was 87% survival after 10 days. Again, larval survival was similar in grooves and holes. However, survival in the plastic drinking straws was higher (Table 1), although it was difficult to locate the larvae in the media to determine survival. Larvae in holes within drinking straws required less frequent transfers — only once a month. McGinnis and Kasting (1962) suggested that the higher survival of larvae within drinking straws might be related to greater chances of the larvae getting injured during transfers. Our data, on the contrary, show that the drinking straws provided a better environment for both larval development and diet quality, as we did not observe growth of microorganisms on the diet medium. In summary, the modifications made to the *S. frugiperda* diet improved the overall quality of the artificial diet. Evaluation is complicated by the fact that fully developed larvae enter facultative diapause for 3 months before pupation.

A total of 36 newly hatched larvae were used to test the *O. nubilalis* diet. A mortality rate of 67% was observed within the first 10 days. All larvae were dead within 20 days, which was attributed mostly to starvation, since there were no signs of diet consumption or tunneling.

We were able to rear *C. cinctus* on an artificial diet for over 60 days, which is five times longer than the survival period of 14 days obtained by Kasting and McGinnis (1958). We were also able to keep alive approximately 18% of the larvae until the termination of the study (60 days). There is a need for further studies of the biology and behavior of *C. cinctus* in order to make further modifications to the diet and improve survival. More research on the environmental variables that may influence larval survival and maturation, as well as on larval nutritional requirements, is necessary. In fact, our research group is currently evaluating the effect of temperature and photoperiod on the onset of *C. cinctus* diapause, for individuals reared on artificial diet.

To mimic field conditions, we kept larvae and diet media under constant darkness inside an environmental growth chamber. The absence of light had no apparent effect on *C. cinctus*

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feeding behavior but might have had an inhibitory effect on the spinning of cocoons and diapause initiation. Effects of light on post-diapause development of *C. cinctus* have been reported (Villacorta *et al.* 1971), but effects on pre-diapause development remain unclear (Holmes 1975). In an attempt to determine the effect of light on diapause initiation of *C. cinctus*, in a previous study we (W.L.M., unpublished data) painted infested wheat stems black and observed no inhibitory effects on diapause initiation.

A better understanding of the interactions between *C. cinctus* larvae and the environment remains crucial to laboratory rearing success. Temperature, humidity, and photoperiod are possibly the most important abiotic factors involved in larval development. Salt (1947) demonstrated the important role of temperature, not only for breaking diapause but also for triggering it. The interaction between temperature and humidity was also discussed (see Salt 1947; Church 1955).

Rearing *C. cinctus* larvae in the laboratory will allow us to perform various bioassays, such as evaluations of larval susceptibility to insecticides and host-plant resistance assays, without having to depend on a short period of time, from late spring to late summer, when larvae are available in the field. Moreover, having larvae readily accessible in the laboratory will make synchronization between host-plant stages and insect stages easier to achieve for other research using plants that are grown in greenhouses.

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