Design and performance of a 96 cell thermally controlled multi-cell instrument

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ABSTRACT

This paper describes the design, construction, and evaluation of a thermally controlled multi-cell instrument containing ninety-six independently controlled cells (designated as the TCMCI-96). Each cell was designed to operate over a temperature range of -20°C to +50°C with accuracy to the setpoint of ± 0.2°C compared to the setpoint requested by the operator at any point within the specific cell temperature cycle. The TCMCI-96 instrument was designed to meet the temperature range and accuracy objectives as well as a number of engineering design objectives to improve overall utility and serviceability when compared to the instrument reported by McLaughlin *et al.* (1985) and a previous one hundred and seventy-six cell version (designated as the TCMCI-176). This paper describes the design and illustrates the utility, flexibility, and accuracy of the TCMCI-96 by presenting data from biological studies conducted within the TCMCI facility at the Saskatoon Research and Development Centre. The temperature accuracy performance objective was not met as the TCMCI-96 achieved a ± 0.3°C accuracy compared to the set point. The temperature range and cycle frequency objectives were met and can be reliably used provided humidity around the instrument is controlled correctly.

RÉSUMÉ

Cette publication décrit la conception, la construction et l'évaluation d'un instrument multicellules à commande thermique contenant quatre-vingt-seize cellules à commande indépendante (appelé le TCMCI-96). Chaque cellule a été conçue pour fonctionner sur une plage de température de -20°C à +50°C avec une précision du point de consigne de ± 0,2°C par rapport au point de consigne demandé par l'opérateur à tout moment du cycle de température particulier de la cellule. L'instrument TCMCI-96 a été conçu pour répondre aux objectifs de plage de température et de précision ainsi qu'à un certain nombre d'objectifs de conception technique visant à améliorer l'utilité globale et la facilité d'entretien par rapport à l'instrument rapporté par McLaughlin et coll. (1985) et à une version précédente de cent soixante-seize cellules (désignée sous le nom de TCMCI-176). Cet article décrit la conception et illustre l'utilité, la flexibilité et la précision du TCMCI-96 en présentant des données provenant d'études biologiques menées dans les installations du TCMCI au Saskatoon Research and Development Centre. L'objectif de performance en matière de précision de la température n'a pas été atteint, le TCMCI-96 ayant maintenu une précision de ± 0,3°C par rapport au point de consigne. Les objectifs de plage de température et de fréquence de cycle ont été atteints et peuvent être utilisés de manière fiable à condition que l'humidité autour de l'instrument soit correctement contrôlée.

KEYWORDS

Thermally controlled instrument, insect development models, germination, thermal-gradient instrument, biological control

MOTS CLÉS

Instrument à contrôle thermique, modèles de développement des insectes, germination, instrument à gradient thermique, contrôle biologique

INTRODUCTION

Laboratory evaluation of biological materials relies on growth environments that can be accurately determined, consistently replicated, and efficiently utilized for a substantial amount of material to facilitate comparative evaluation and statistical analyses. McLaughlin *et al.* (1985) summarized numerous efforts to replicate natural growth environments for weed seed germination. In response to the need for more accurate temperature control, flexible temperature cycling over a biologically meaningful range of temperatures, and replication of multiple temperature conditions, McLaughlin *et al*. (1985) successfully developed a seed germinator containing 100 independently controlled cells within a single instrument. The seed germinator design hardwired each cell with replaceable reference resistor banks to enable each cell to hold a static temperature or cycle through up to six (6) temperatures within a 24 h period. The temperature cycling, controlled by a sizeable motorized switch, was designed to approximate a normal diurnal temperature fluctuation experienced by the biological material placed within the germination cells.

In 1995, a new 176 cell thermally controlled multi-cell instrument (designated as the TCMCI-176) was designed and constructed by the Engineering Section of the Swift Current Research and Development Centre to replace the 100-cell instrument designed by McLaughlin. This design was the prototype version of the TCMCI-96 described within this paper (Fig. 1) and encompassed several essential features, including:

- Personal computer (PC) operator interface and temperature control using an IBM-AT class DX-66 PC;
- Software, rather than hardware, calibration of all temperature sensors to improve operator efficiency and experimental accuracy;
- Wider temperature range of operation (0ºC to 40ºC) with improved temperature control accuracy (± 0.2ºC); and
- Three implemented control strategy options: constant temperature, step-change for temperature, and true sine-wave diurnal temperature fluctuation.

The TCMCI-176 was removed from the Saskatoon Research and Development Centre, refurbished, and moved in March 2014 to the Agassiz Research and Development Centre located in Agassiz, British Columbia (Figs. 1 and 2). The developed TCMCI-96 targeted the replacement of the TCMCI-176. Three TCMCI-96 units are currently operating at the Saskatoon Research and Development Centre. The remaining sections describe the design and validation of the TCMCI-96.

Researchers using the Saskatoon TCMCI-96 and TCMCI-176 facilities have documented their work in 21 scientific publications and at 16 scientific conferences and counting. The following are examples of science conducted using these unique instruments:

Entomology Researchers have performed several studies involving integrated pest management (IPM), population dynamics, and climate change. The TCMCI-96 has been used to conduct entomological studies related to survivorship, development, reproduction, and feeding of insects at various life stages (e.g. Liu *et al.* 2019). Entomologists have conducted cold stress studies to analyze the impact of cold and heat stress on the mortality of both pests and beneficial species. These include an ongoing project studying the cold and heat tolerance of the natural predators of the diamondback moth (*Plutella xylostella*). Insect pathology studies with spinosad investigated fungal pathogen development on insect hosts (Benjamin 2002; Elliott *et al.* 2007). Finally, Elliott *et al.* (2007) also investigated the efficacy of registered insecticides under different thermal conditions. These studies and others provide significant contributions to developing more accurate bioclimatic models for insect distribution and phenology models for insect development.

Biopesticide Research The TCMCI Facility has been instrumental in several aspects important to this area of agricultural development, including a) characterization of growth characteristics of biopesticide organisms; b) evaluation of non-indigenous biopesticide organisms, including 'habitat matching'; c) environmental fate and survival of biopesticide organisms (e.g. Hanson 2008;

Fig. 1. TCMCI-176 installed at Saskatoon, SK.

Fig. 2. TCMCI-176 installed at Agassiz, BC.

Hanson *et al.* 2008); and d) evaluation of biopesticides under variable temperature regimes (e.g. Peng and Boyetchko 2006). These data have contributed to defining the biological and ecological limits of growth, which are parameters required by the Pest Management Regulatory Agency for registering biopesticides. The data collected from the TCMCI Facility have allowed for early risk-assessment decisions, increasing the safety of researchers, technical staff, and the general public exposed to fungi or other biopesticide organisms in the laboratory and field.

Integrated Pest Management of Plant Pathogens Two groups successfully used the TCMCI Facility to quantify soil temperature impacts on seedling infection by soil-borne pathogens in pulse crops (e.g. Hwang *et al.* 2000; Chang *et al.* 2004, 2008). These studies involved external collaborators from Alberta Agriculture and Forestry and the Alberta Research Council. Plant scientists validated the relationships determined from these studies using field trials. These field trials helped develop recommendations for seed treatments and optimum seeding dates to manage plant soil-borne pathogens. Similarly, now concluded studies of seedling blights on canola and their planned matching field trials are excellent demonstrations of the instrument's usefulness.

Weed Ecology The TCMCI units have been used to develop mathematical models and improve our general understanding of the seed germination and shoot emergence processes concerning temperature (e.g. Oryokot *et al.* 1997; Roman *et al.* 1999; Shrestha *et al.* 1999). Results from seed germination experiments on the instrument are complementary to detailed monitoring of emergence in the field. The field trials also investigate the effects of the tillage system, seeding practice, soil type, and rates of nitrogen mineralization in addition to temperature and moisture stress. This research is used in bio-economic models for weed population thresholds and incorporated into decision support systems for weed management.

Visitors and collaborators from several other scientific organizations and universities have expressed interest in the TCMCI concept and design on many occasions, including during Saskatoon Research and Development Centre tours, collaborative research projects with collaborators external to AAFC, and following presentations at scientific conferences. However, as the design is proprietary to AAFC, no commercialization plans have been made at this time. Instead, Saskatoon Research and Development Centre staff have formed strong collaborative relationships with these scientists, thus broadening this technology's utility.

OBJECTIVES
The experience gleaned from designing, constructing, and operating the TCMCI-176 was invaluable when the Engineering Section personnel met with Saskatoon Research and Development Centre personnel to determine performance objectives for the new TCMCI-96 design. The objectives of this work were to develop a TCMCI-96 instrument that met the following performance objectives:

1. Enhanced temperature range capability (-25°C to +50°C) while maintaining accuracy ($\pm$ 0.2°C) between actual and setpoint temperatures at any point in time within a control cycle;
2. A consistent temperature within cells to ensure a consistent and accurate growth environment within each cell; and
3. Multiple temperature cycles within a 24 h period.

The stakeholders also established the following engineering design objectives for the TCMCI-96:
1. Deeper cells (100 mm and 120 mm depths) to improve the utility of the TCMCI-96 for seedling production, insect rearing, and experiments requiring sample volume;
2. Ninety-six (96) cells to facilitate installation of the device within each facility while allowing for adequate numbers of cells to meet statistical requirements for a wide variety of studies;
3. Increased software functionality, including control over cycle type, time, and duration and display of real-time and historical cell data at all times during instrument operation;
4. Improved operator interface to include visual and audio alarms for individual cell deviation, on-the-fly cell activation and deactivation, and multiple user interactions with cell block protection;
5. Networked operating software to allow monitoring from an offsite location, instrument interaction, data storage in a secure, redundant setting within the local area network (LAN), and network and external alarms to alert users when a critical performance attribute is outside user-defined limits;
6. Simplified cell mounting system to facilitate repair of thermoelectric devices and temperature sensors as required; and
7. An overall improved design to facilitate ease of construction and lower the total cost.

MATERIALS AND METHODS
General Layout and Nomenclature
The design of the TCMCI-96 has two pods, each containing three modules of sixteen cells per module (Fig. 3). Each cell has a discrete number from 1 to 96. The design has a binary aspect due to the limitations of the instrumentation and control devices used for the instrument. The sixteen cell modules also facilitated the ease of construction and assembly. The modularity allows operators to repair a cell or other malfunction in a single module without disrupting the other modules tasked with an experiment. The coolant required for each row of four cells in a module is provided from a common supply rail in a countercurrent flow pattern through two parallel coolant tubes to ensure consistent coolant temperature underneath each cell.

For each cell, temperature sensors connect to an interface board and NI^{TM} AMUX-64T multiplexing board located in a sensor interface panel at the end of each pod, separating the measurement instrumentation from the high
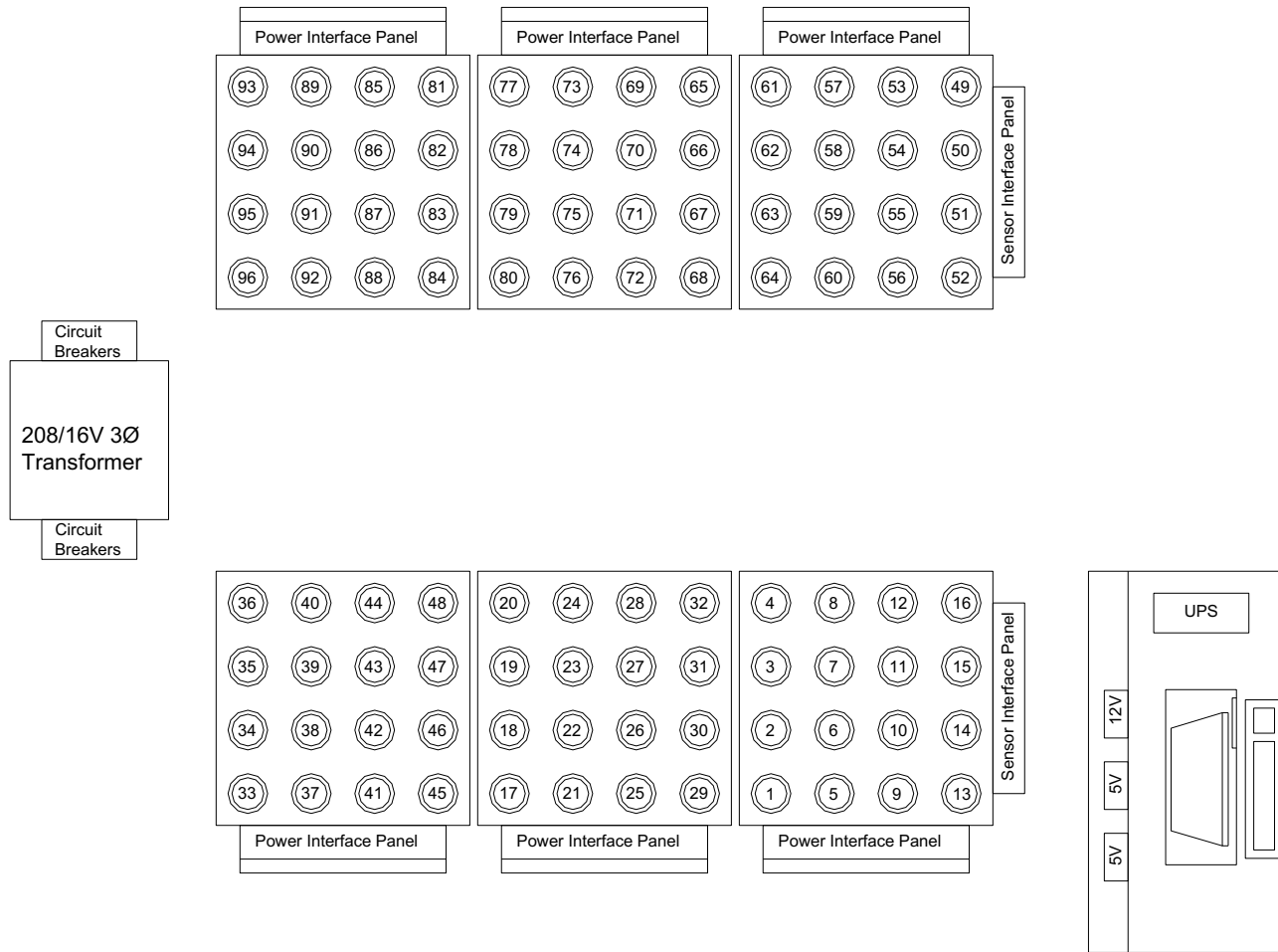
Fig. 3. Power interface panel (1 of 6 required for the TCMCI-96).

current supplies and control relays. A power interface panel located below each module (Fig. 4) contains:

1. Nominal 12V alternating current (AC) 3 phase (3Ø) power connection from the transformer, and the 3Ø bridge rectifier and capacitor system used to generate the ± 12V direct current (DC) power required for the thermal electric devices that heat and cool each cell;

2. "Very-Fast-Blo" fuses to protect the power control board and its components;

3. Custom designed power control board;

4. Microchip™ PICDEM PIC18 Explorer Board (PIC18 board); and

5. Connections including serial communications from the control computer, 5V DC for the operation of the power control board, and 5V DC for the operation of the PIC18 board.

A custom-designed 33 kVA 3Ø AC transformer built by Hammond Transformers converts the building's 3Ø AC power into approximately 12V RMS 3Ø AC power. The design includes an additional Neutral wire to the cables transferring this AC power to each module. A 3Ø bridge rectifier converts the 12V RMS 3Ø AC into ± 12V DC with the Neutral providing the ground plane between these two voltages in each module. Two Mallory 40V 40,000 uF capacitors (one connected between the +12V bus and Neutral and one connected between the -12V bus and Neutral) help shield switching noise from the power control board. The +12V DC and -12V DC are connected to separate aluminum bus bars where "Very-Fast-Blo" 8 amp fuses are placed on the custom-designed power control board between the bus bars and the individual cell connections. The Neutral is connected directly to the pair of thermoelectric devices with heating and cooling power supplied by the +12V (cooling) or -12V (heating) bus via the control logic and the power control board.

The Analog Devices™ AD590K temperature sensors are powered through a custom interface board (one per module) by a 12V DC linear power supply that reduces the potential of signal noise and improves temperature
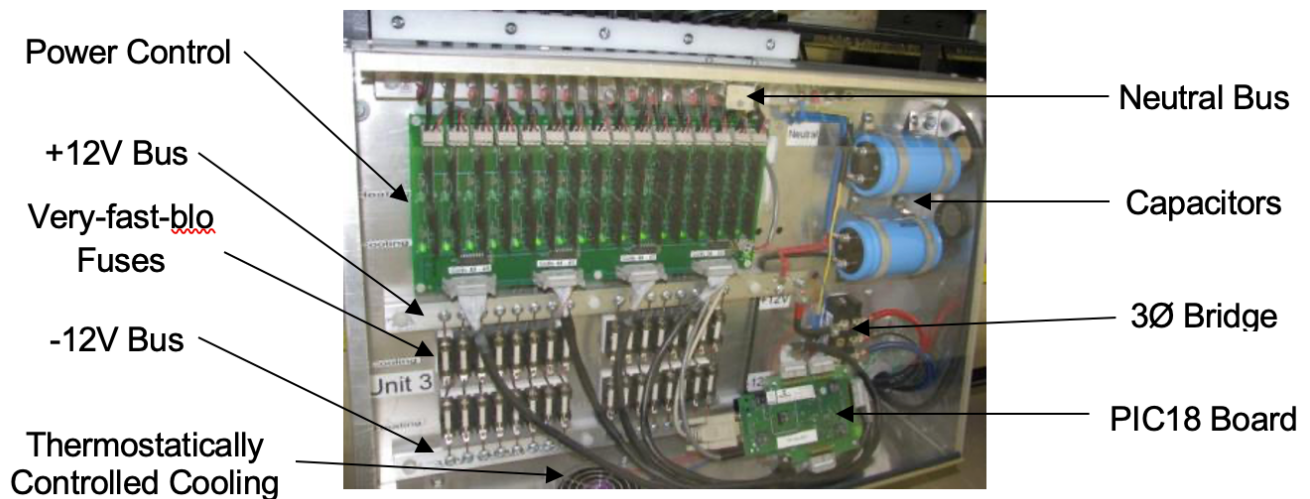
**Fig. 4. Power interface panel (1 of 6 required for the TCMCI-96).**

sensitivity compared to a 5V DC linear supply. This custom interface board converts the current versus temperature signal from the AD590K sensor to a voltage versus temperature signal measured by the NI™ AMUX-64T board. A 5V DC linear supply is used to power the NI™ AMUX-64T board instead of the internal computer power supply to reduce signal noise and reduce strain on the control computer power supply. The NI™ AMUX-64T and the custom interface boards are contained within the sensor interface panel located at the end of each pod of forty-eight cells (Fig. 5). An additional custom interface board in the left-hand sensor interface panel converts the coolant water input and output temperature sensor signals.

The power control board and the PIC18 board located within each power interface panel are powered by a single 5V DC switching power supply. All of the power supplies mentioned and the PC with the monitor used for interface and control of the TCMCI-96 are supplied by an uninterruptible power supply (UPS) large enough to carry the combined load for approximately ten minutes. This interval is more generous than needed to maintain power until the emergency power system within the building engages during power interruptions caused by monthly building maintenance checks or loss of external power service.

Coolant System

When the thermal electric devices provide heat to any given cell, the opposite side absorbs heat from the heat exchanger and the coolant flowing within. This configuration is the most efficient operation of these devices because the heat generated includes both the Pelletier effect and the current (I^2R) heating from the power going through each device. When the thermal electric devices absorb heat from any given cell, heat is transferred into the heat exchanger and coolant. For any given difference between the cell and coolant temperature, the amount of heat transferred to the coolant when cooling the cell is greater because the thermal electric devices must overcome the I^2R heating and the heat absorbed from the cell's environment. The installed thermal electric devices have a maximum temperature reduction of
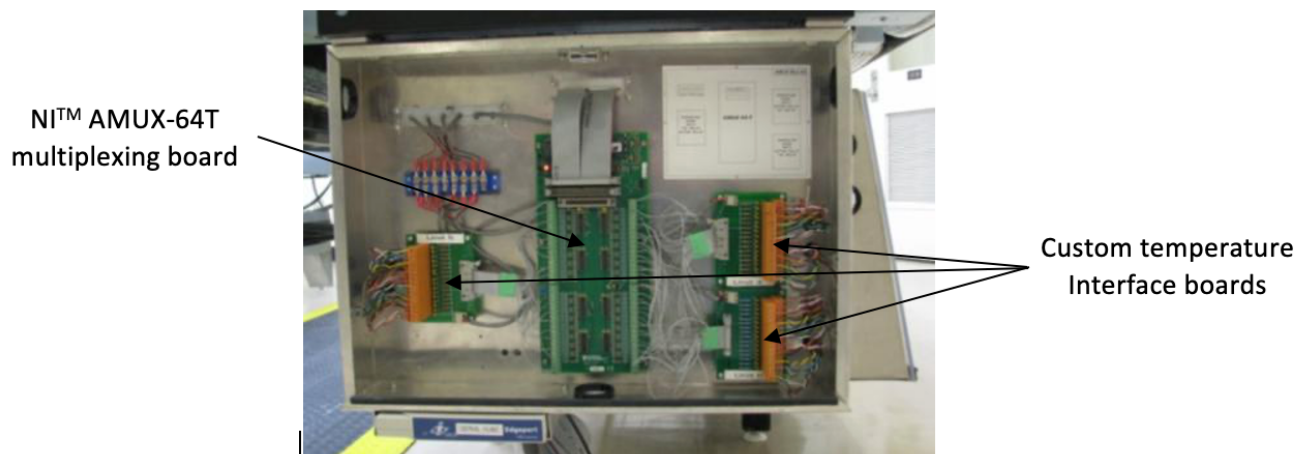
Fig. 5: Sensor interface panel without coolant temperature conversion board.

Fig. 6: 100 mm cells installed on heat exchangers for a 16-cell module.

30°C below the coolant temperature. Therefore, the instrument must maintain the coolant temperature between 8°C and 10°C for efficient operation and temperature capability. The heat exchangers used are custom aluminum extrusions provided by the Paramount Extrusion Co. of Paramount, CA. These exchangers connect to aluminum manifolds on each pod, with both pods of each TCMCI-96 connected to a pump and coolant supply tank. The pump is connected to a 220 V 1Ø UPS to ensure coolant flow during a power interruption. The use of a UPS and a coolant supply tank is necessary to ensure the coolant temperature, and coolant manifold pressure remains within operational safety limits during a power interruption. Otherwise, the computer will shut down the TCMCI-96 to protect the instrument and potentially cause a loss of an entire set of experiments.

The coolant fluid used is an industrial glycol designed to prevent oxidation and corrosion of coolant system elements. The coolant is chilled by a 17.5 kW (5 Ton) Chillers Inc. air to liquid chiller located in an adjacent mechanical room. There is no internal coolant fluid source within the building. This chiller provides adequate capacity for the entire suite of three TCMCI-96 units deployed at the Saskatoon Research and Development Centre.

Cell Design

The heart of the TCMCI-96 is the cell that contains the experimental material under evaluation. As noted in the Objectives, the scientific teams requested the design and construction of two different TCMCI-96 units, one having cells with a depth of 100 mm, the other with a 120 mm cell throughout the instrument. Note that the resulting modular design of the TCMCI-96 could accommodate both cell depths within a single TCMCI-96 if necessary. Each module can contain 16 cells with a uniform height different from a neighbouring module with 16 cells of a second height. Figure 6 identifies vital components in a module of sixteen 100 mm deep cells, including temperature sensors and thermal electric device connections, and Fig. 7 is a schematic diagram of the 120 mm cell.
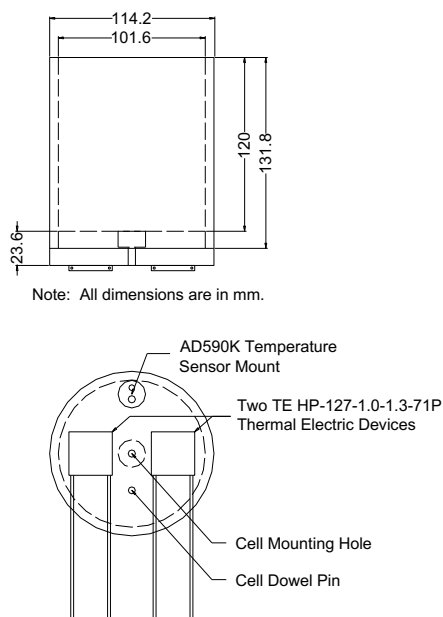
Two TE Technology Inc. HP-127-1.0-1.3-71P thermal electric devices, wired in parallel, are placed beneath each cell. Each pair of the thermal electric device receives approximately 8 amps of ±12V DC power from the power control board, depending on whether the cell is being heated or cooled. Analog Device's AD590K temperature sensors are custom mounted to take the temperature measurements. These devices require a 10kΩ precision resistor to convert the current generated by the AD590K proportional to temperature to a voltage (0 – 6V DC). The resistors are mounted on a custom interface board so that each interface board has connections and resistors for sixteen cells from each module. The individual outputs for each cell from this board connect to the NI™ AMUX64-T for multiplexing the voltages back to the NI™ 6036E analog to digital conversion (A/D) board contained within the control PC.

Heating and Cooling Control

Custom-designed boards utilizing Crydom™ CMX60D10 MOSFET DC solid state relays were designed to use a pair of relays for each cell to connect their thermal electric devices to either +12 V or -12V DC. The onboard driver chip provides the necessary voltage and current to switch the relays based on the PIC18 board's output. The fuses for one or both of the MOSFET relays will fail before a catastrophic relay failure could occur during accidental electrical shorts or if a software error requests both heating and cooling simultaneously.

Control Hardware and Software Description

A PC operating with Windows 10 Pro™ is used as the control and interface computer for each TCMCI-96 instrument. Mr. Alan Jones, formerly of the AAFC Ottawa Research and Development Centre, designed and created the PC control software using the Delphi platform. The program uses NI™ drivers for the PCI-6036E A/D board, AMUX-64T multiplexer board, custom interface software for the PIC18 board located in each power interface panel, and drivers for the EdgePort™ USB port replicator used to

Note: All dimensions are in mm.

AD590K Temperature Sensor Mount

Two TE HP-127-1.0-1.3-71P Thermal Electric Devices

Cell Mounting Hole

Cell Dowel Pin

Fig. 7. Schematic drawing of 120 mm cell with base and thermal electric devices.

connect the computer to the PIC18 board required for each module.

Thermal electric device response to heating is approximately three times faster than cooling. This asymmetry requires different PID (proportional- integral-differential) coefficients for each condition. Extensive experimentation determined the PID coefficients for a single TCMCI-96 and are used in all other TCMCI-96 instruments regardless of cell depth. Using the PID control strategy and the current set of coefficients allows the instrument to meet $\pm$ 0.3˚C temperature control accuracy. The PIC18 board contains a custom program that utilizes the PID coefficients and the desired temperature setpoint from the computer to send control signals to the power control board relays to heat or cool each cell to the desired temperature set point. The control software provides for the following:

1. Simple calibration of the instrument. The user allows the instrument to reach temperature stasis within the room over a 24 h period, then commences the "Calibration" subroutine and enters the known temperature measured within the room using a temperature standard. The software calibrates each temperature sensor accordingly and records the appropriate offset required for the NI[TM] measurement system. A gauge on each coolant system's input and output manifolds provides known values to calibrate the pressure sensors. If necessary, voltages from each power supply are adjusted on each source to their desired voltage using a Fluke multimeter and then entered into the "Calibration" subroutine.

2. Independent temperature control for each cell. The user needs to choose the type of cycle: constant temperature, sine wave temperature cycling with desired endpoints and frequency (up to 6 cycles per 24 h period), or step-change temperature control with endpoints and frequency of change.

3. Track the number and locations of cells assigned to each user or experiment. If a given cell fails to operate within the required control parameters, it can be disabled, and the software will select an unused cell as a replacement on the fly.

4. Operator monitoring of cell performance. Cell data is reported to the operator by a visual interface that uses colour-coded circles located on the screen to represent the instrument layout. Depending on the operator's screen selection, detailed cell and manifold temperature, cell set point, heating or cooling status, instrument performance history, and alarm condition are available.

5. Fail-safe operation. Alarms or alerts occur when cell performance is outside acceptable parameters. The software is configurable to shut down the cell and message the operator via telephone, modem, or SMS text message through the LAN. If the coolant temperature exceeds the setpoint or the coolant pressure exceeds the specific set point, the entire unit will alarm at the first level. After a user-defined delay, the unit will shut down when the limits are exceeded at the second limit level to prevent damage to the instrument. The system monitors every voltage supply to ensure that the 5V and 12V supplies operate within an acceptable voltage range. A safe shutdown will occur if either supply voltage strays outside the operator's established acceptable levels.

System Testing

A series of tests were conducted to ensure the TCMCI-96 instrument met the performance criteria for operational limits. First, programs were created and run to determine that all cells could reach and sustain temperatures between 30ºC and -25ºC. Second, the temperature cycling rate limit from hot to cold was determined to set the operating limits for the number of achievable temperature cycles within a 24 h period. A 3.5 kW (1.0 ton) air to liquid chiller provided the cooling capacity required for this series of tests, maintaining a coolant temperature of 10ºC. Seventeen different cells (of the 96 available) were randomly selected for testing. This testing process bypassed the normal PID software control to ensure 100% duty cycles were maintained during heating and cooling until the desired set points were reached. The cell temperatures were initially calibrated, then 17 test cells were warmed to 30ºC before being cooled to -25ºC. The time required for the temperature in each cell to fall to -25ºC was recorded, as was the time needed to reheat the cells to 30ºC and 50ºC. Fluke[TM] data loggers were used to measure temperatures inside the cells for these two tests.

Once installed at the Saskatoon Research and Development Centre, a series of four tests were conducted to evaluate the consistency of the cells in holding five setpoint temperatures (-10, 0, 10, 20, and 30ºC). Temperature recordings were made using Onset
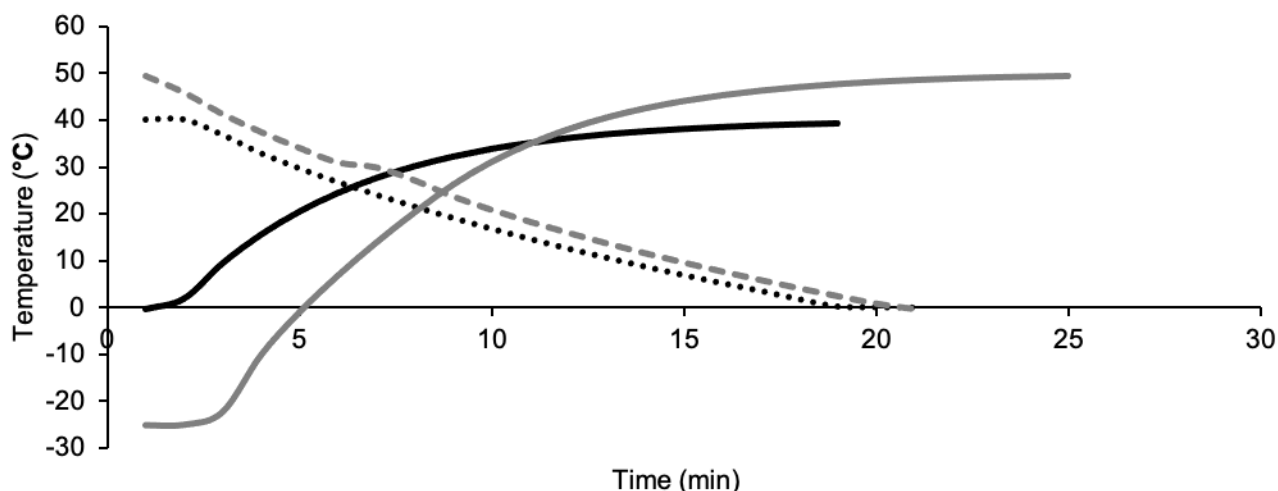
Fig. 8. Representation of the mean time for typical TCMCI cells to change temperature (°C), represented as performance curves.

temperature probes placed inside Petri dishes lined with moistened filter paper to ensure consistent humidity conditions. Temperature recordings were made every minute for 24 h, and mean, maximum, and minimum temperatures were calculated for a 60-minute interval at approximately the same time of day, at least 4 h after the cells stabilized at or near the setpoint temperature. The data were used to determine the accuracy of the cells by calculating the standard deviation and standard error around the mean cell temperatures (which were close but not equal to the setpoint temperatures).

Temperature stratification in the deep cells of the TCMCI-96 unit was quantified using Onset probes in cells at set points of 0, 4, 10, 20, and 30°C. For comparison, temperature stratification was also tested in two growth cabinets at set points of 4 and 24°C. Petri dishes with Onset probes were placed on the top, middle, and bottom shelves in the growth cabinet. On each shelf in the growth cabinet, Petri dishes were in the centre of the shelf or adjacent to the eastern wall of the cabinets. The temperature in the cells of the TCMCI-96 unit and the growth cabinets was recorded during four 60-minute intervals once the cells/cabinets had reached their assigned setpoint temperature.

Finally, to investigate the effect of temperature stratification in 120 mm deep cells of the TCMCI-96 on biological material, we conducted tests on the germination of canola seed (*Brassica napus*). Canola seed germinates best within a narrow range of temperature (e.g., 15 to 20°C), at which germination occurs quickly (Kondra et al. 1983). Therefore, consistent seed germination across an individual petri dish and between stacked Petri dishes indicates that biologically consistent temperatures are maintained throughout a typical TCMCI cell and across TCMCI cells set to the same temperature. Five Petri dishes containing seeds of *B. napus* were stacked in randomly selected 120 mm deep cells, with Dish 1 at the bottom of the cell and Dish 5 at the top of the petri dish stack. The investigation tested a total of seven setpoint temperatures (10, 12, 15, 18, 20, 22, and 30°C), with four replicates for each setpoint. Experimenters recorded the time required in each replicate for 80% of the seeds in each dish to germinate.

RESULTS AND DISCUSSION

All 96 cells in each TCMCI-96 unit could reach and sustain setpoint temperatures between 50°C and -25°C. Fig. 8 is an example of the temperature performance of a typical cell using full PID software control under typical laboratory operational circumstances. The mean time required for 17 cells to transition from 30°C to -25°C was approximately 45 minutes (Fig. 8). Less time was needed to warm the cells from -25°C to 30°C (mean time about 10 minutes), and approximately 20 minutes were required for cells to warm from -25 to 50°C (Fig. 8). Despite the increase in time needed to heat and cool from the cold and hot limits, the TCMCI-96 data demonstrates that a 6 cycle per day requirement can be accomplished, which meets the objective for multiple temperature cycles within a 24 h period. This compares to the single cycle per day provided by the instrument developed and reported by McLaughlin *et al.* (1985).

The accuracy of the cells, represented by the standard error (Fig. 9) and standard deviation (Fig. 10) around each set point's mean temperature, is outside of the desired ± 0.2°C performance goal described in the objectives. However, at ± 0.3°C to ± 0.5°C, the performance of the instrument is well within the performance range necessary for its intended biological applications. This performance compares well against the performance reported for the unit described by McLauglin *et al.* (1985), where the mean accuracy of all cells was ± 2°C of the setpoint temperature with 93.2% of all measured cells within ± 1°C of set point and 57.4% of all measured cells at setpoint temperature.

Up to five standard-sized Petri dishes can be stacked in the 120 mm-deep cells of the TCMCI-96, allowing
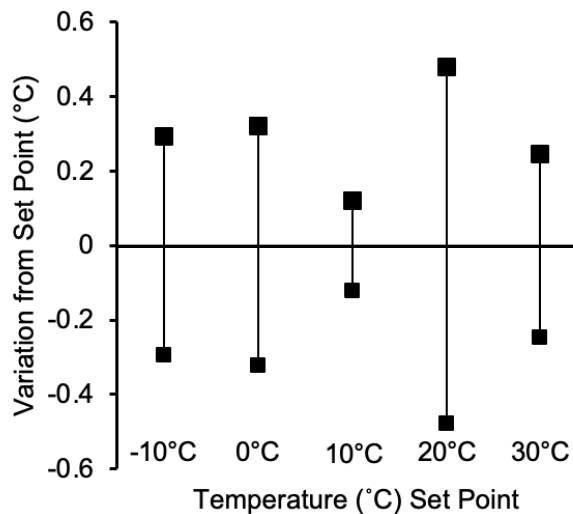
Fig. 9. Standard deviation (±) of the mean cell temperature from five temperature setpoints; the horizontal axis represents the mean temperature in the replicate cells during the 60-minute interval used for calculations (the mean was close to, but not exactly equal to the set point temperature).
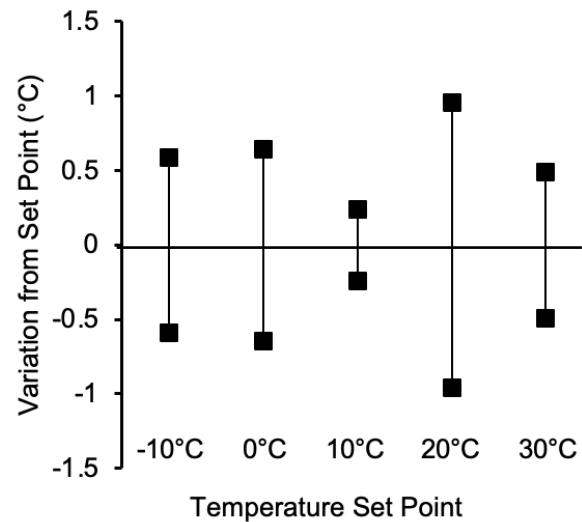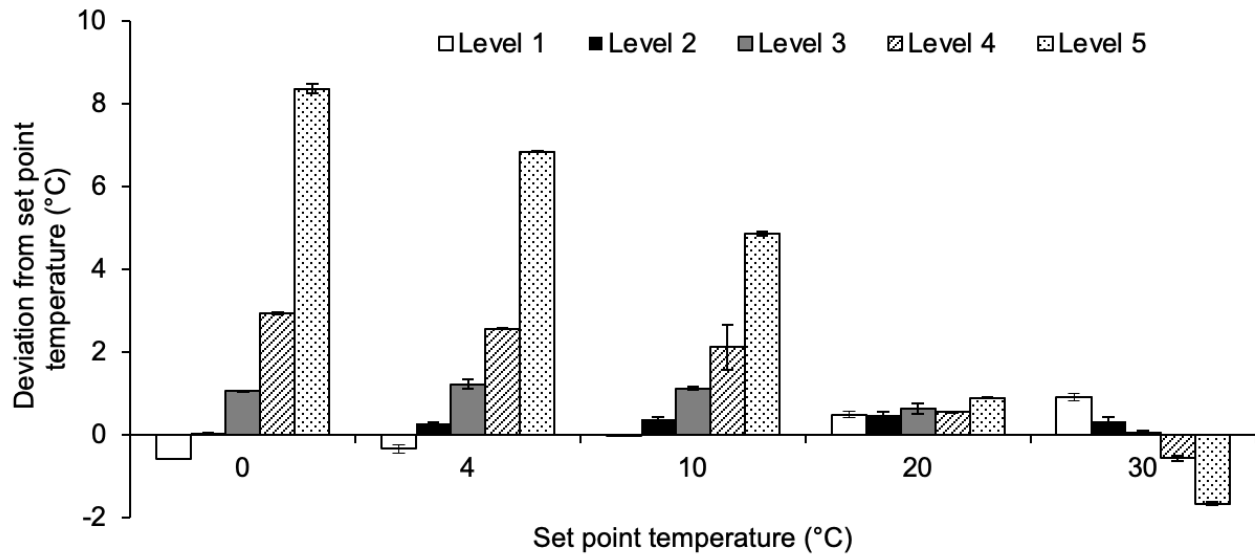

Fig. 10. Temperature deviation from two set points (4 and 24°C) on the top, middle, and bottom shelves of growth cabinets when dishes were beside the east wall and at the centre of each shelf.

researchers to design complicated experiments while maximizing experimental units. It is possible to use growth cabinets similarly, but the temperature stratification is a confounding factor that is rarely well-controlled. For some statistical purists, replicating an experiment within a single growth cabinet is considered "pseudo-replication." The variation around the set point in the growth cabinets was less than expected based on experience with older technology (Fig. 11). However, the shelf height and location of dishes on a shelf influenced the temperature recorded by the Onset probes, indicating the need to move experimental units between shelves and locations on the shelves during experiments to control temperature differences inside growth cabinets.
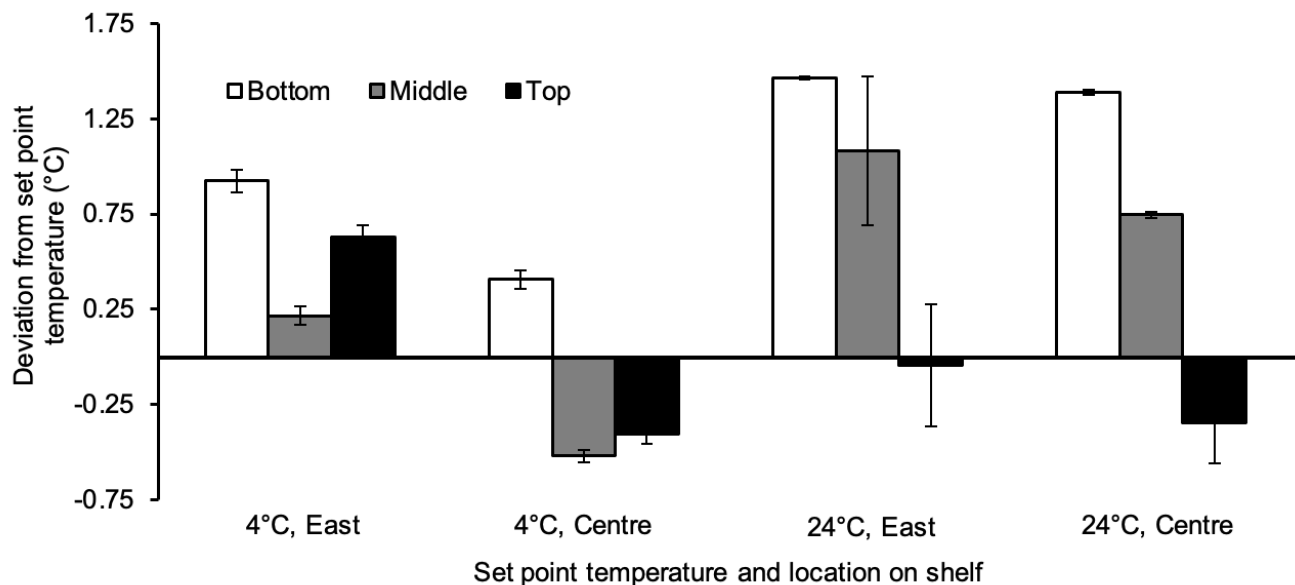

Fig. 11. Temperature deviation from two set points (4 and 24°C) on the top, middle, and bottom shelves of growth cabinets when dishes were beside the east wall of the cabinet or set at the centre of each shelf.

Fig. 12. Temperature deviation from the setpoint, measured by Onset data loggers inside petri dishes in stacks of five dishes within TCMCI-96 cells.

Temperature stratification also occurred in the TCMCI-96 cells (Fig. 12). Here, the bottom three Petri dishes (Levels 1 – 3) in the stack experienced the least temperature variation, approximately ± 1°C or less. Dishes at Levels 4 and 5 experienced more deviation from the setpoint, especially at cooler temperatures (Fig. 12). Temperatures near the top of the TCMCI-96 cells are more difficult to control due to their proximity to ambient room temperature and lack of insulation at the cell opening. Using two layers of watch glasses to cover the cells can reduce temperature variation (after McLauglin *et al.* 1985). Users can regularly rotate Petri dishes within the cells to ensure equal exposure of all biological material to temperature stratification effects. Limiting stacks to three dishes will also reduce stratification effects. These measures are included in the standard operating procedure developed for experiments using stacked cells in the TCMCI-96 units.
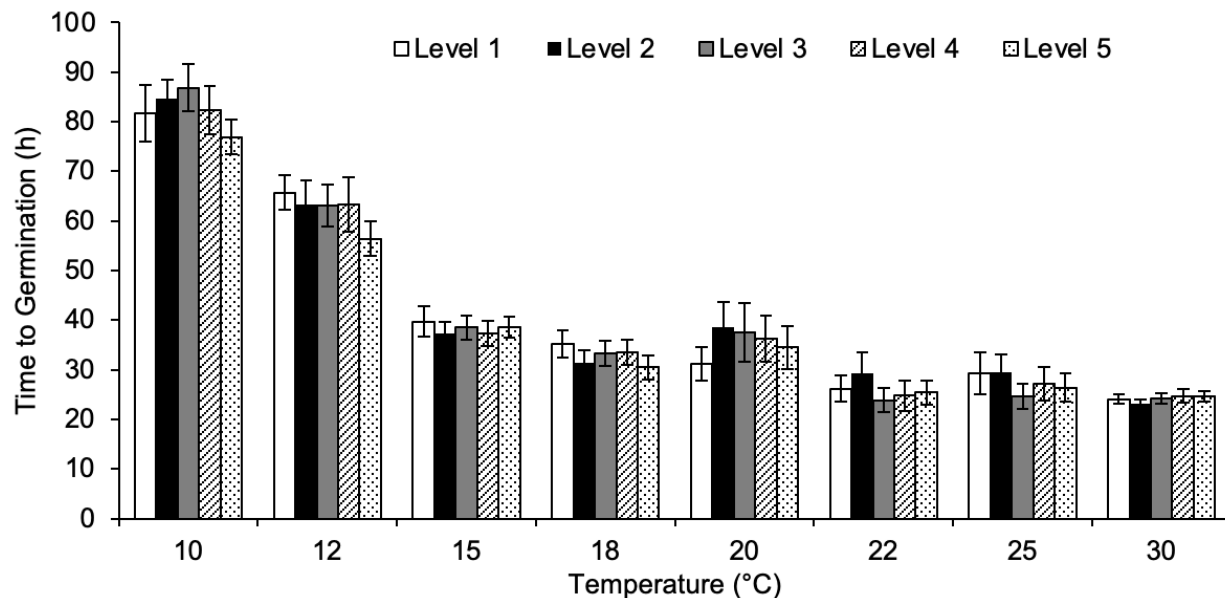
Fig. 13. Comparison of *Brassica napus* generation time for seeds inside stacked petri dishes (five levels) at eight temperature set points.

The difference in the time to 80% germination between petri dish levels within cells was largest at the coolest temperatures (Fig. 13). For example, at 10°C, it took, on average, 10 h longer for Level 3 to reach 80% germination than Level 5, which was on top of the stack. The top-level was likely warmer than the set point of 10°C due to its proximity to ambient room temperature conditions. When the cell set point was above ambient room temperature, seeds in Levels 4 and 5 germinated more quickly than seeds in Levels 1 and 2, but more slowly than seeds in Level 3 (although the differences do not appear to be statistically significant). Thus, at warmer experimental temperatures, the effect of stratification in the TCMCI-96 cells was reduced relative to colder experimental temperatures. When experiments require stacking Petri dishes or other experimental media inside the TCMCI-96 cells, standard operating procedures require regular rotation of the stacked plates to control and limit the impact of temperature stratification in TCMCI cells on experimental results.

CONCLUSIONS

The TCMCI-96 essentially meets the performance and engineering objectives described in the Objectives. The temperature accuracy performance objective was only partially met as the demonstrated accuracy of $\pm$ 0.3°C between actual and setpoint temperatures at any point in time within a control cycle was outside the performance goal of $\pm$ 0.2°C. However, the accuracy of $\pm$ 0.3°C is within reasonable biological limits and exceeds the temperature control performance reported by McLaughlin *et al.* (1985). The engineering design objectives were met in all respects.

One weakness of the TCMCI-96 design is the custom-designed software used for the PC, NI[TM] boards, and the PIC18 single board computers. This custom software limits users' ability to change the software or replace obsolete devices that fail. However, the user interface and control excellence have set an example for any future design to emulate. Future instruments of this type will require a substantive change in the control and measurement software and hardware used to interface between the device and the control PC. The authors recommend that off-the-shelf programmable devices using software such as CODESYS, Visual Basic[TM], Lab View[TM], Linux[TM] or other common control operating systems be sourced and deployed. When drafting this manuscript, the authors noted that programmable logic controllers could measure the sixteen individual temperatures and control the thirty-two relays within a TCMCI-96 module. If such suitable devices were found and the appropriate software developed for integration with a control PC, new instruments could be designed and constructed to accommodate as many 16-cell modules as needed for the particular installation, with each module controlled from a single PC as is currently the case.

One of the requirements for the TCMCI-96 installation is a reduced (below 35%) relative humidity within the laboratory space when cell temperatures are below 8°C. This humidity requirement is to prevent condensation from accumulating near the bottom of the cell, a condition that can affect the cell temperatures, thermal electric device connections, and operational stability. The Saskatoon Research and Development Centre installation placed dehumidifiers within the two laboratories. The dehumidifier output was directed across the cells' bottoms to reduce the likelihood of condensation and maintain a dry condition near the critical thermal electric device and sensor connections. Additionally, sealant was applied to the temperature sensors from the sensor to the sensor connector. Future design installations or variations may utilize prebuilt sealed sensors and watertight connections.

Three TCMCI-96 instruments are in constant demand and use at the Saskatoon Research and Development Centre by Biological Control, Entomology, Oilseed Breeding, and Plant Genetic Resources programs. Other programs are interested in developing experiments to utilize the TCMCI-96 to answer their research areas' questions. The initial TCMCI-96 has been in operation since 2012, with the last two instruments installed and operational as of mid-2014. The total, parts-only cost of each TCMCI-96 is estimated to be C$55,000.00 in 2018 funds.

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