**Title: Enhancement of Bramble Production in the Southeastern U.S. Through Micropropagation, Virus-Indexing, and Field Evaluation for Trueness-to-Type**

**Progress Report**

**SRSFC Project #2008-06**

**Research Proposal**

**Principal Investigators:**
Dr. Zvezdana Pesic-VanEsbroeck
Department of Plant Pathology, North Carolina State University,
Raleigh, NC 27695-7616
zvezdana_pesic@ncsu.edu
Dr. James Ballington
Department of Horticultural Science, North Carolina State University,
Raleigh, NC 27695-7609
jim_ballington@ncsu.edu
Dr. Gina Fernandez
Department of Horticultural Science, North Carolina State University,
Raleigh, NC 27695-7609
gina_fernandez@ncsu.edu

**Objectives:**

1) To develop protocols for *in vitro* propagation of bramble (blackberry and raspberry) cultivars grown in the southeastern U.S.
2) To maintain virus-indexed *in-vitro* Nuclear Stock plants of selected bramble cultivars and advanced selections in the repository in the Micropropagation Unit and Repository (MPUR) at NCSU.
3) To evaluate micropropagated and virus-indexed bramble plants for trueness-to-type.

**Justification:**

Raspberries are potentially a very high-value crop, but they are also one of the most difficult crops to grow in North Carolina and the Southeastern U.S. In North Carolina, commercial raspberry production is concentrated in the western part of the state, in the mountains and in the foothills. Raspberries are not commonly grown in the piedmont and coastal plain areas because most raspberry cultivars are poorly suited to warm temperatures in those areas. New raspberry cultivars that are better adapted to the climate in these areas are being developed by NCSU researchers.

Propagation of raspberry plants by stem cuttings is difficult and inefficient, and most breeding programs are using meristem-tip culture techniques and apical meristems to

produce *in vitro* plants. Also, meristem-tip culture has been used successfully for elimination of plant viruses and other plant pathogens that are commonly found in vegetatively propagated blackberry and raspberry plants and have been known to cause economically important diseases. Differences in the genetic make-up of blackberry and raspberry cultivars and advanced selections require certain modifications of standard tissue culture media and growth conditions to produce viable *in vitro* plants. The MPUR at NCSU maintains a repository of blackberry and raspberry cultivars grown in the Southeastern U.S., and is the source of micropropagated, virus-indexed, true-to-type certified planting stocks.

**Methodologies and Results:**

**Tissue Culture Protocol for *Rubus***

**A. Collection of plant material**

Blackberry or raspberry primocanes are collected from pot-grown greenhouse plants and/or field plants using sterile razor blades, placed in plastic bags, labeled and stored at 4 C. Shoot tips are trimmed to 1-1.5 cm, placed in a beaker and washed in running water for 5 min and then surface sterilized for 10 min in 100 ml of a solution consisting of 0.79% w/v Sodium Hypochlorite and five drops of Tween-20. It is necessary to stir plant material constantly to facilitate surface contact. Shoot tips are transferred and held in deionized sterile water until meristeming.

**B. *In vitro* culture**

**Stage I – Establishment**

After surface sterilization, shoot tips are transferred to a Bioflow laminar vertical flow cabinet and rinsed three times in sterile deionized water for 5 minutes each. Shoot tips are trimmed of all but 1-2 primordial leaves enclosing the dome. Apical meristems consisting of a dome and 1-2 primordial leaves are 0.1-0.5 mm long. Meristems are transferred into 20 x 150 Bellco culture tubes containing 10 ml of Murashige and Skoog (MS) PGR-free liquid initiation medium and placed on a bridge made of filter paper. Meristems are maintained for five to seven days in this medium under bright white fluorescent lights at 24-27 C and the 16-h photoperiod. The same medium is used for establishment of blackberry and raspberry explants.

**Stage II - Proliferation**

Two formulations are currently used for *in vitro* culture of *Rubus* genotypes in the MPUR. MS is used for blackberry proliferation and MS or Anderson's Raspberry Medium are used for raspberry proliferation depending on the genotype.

Meristems are transferred to either MS or Anderson's Raspberry Medium in Magenta boxes (50ml/box), placed under the same conditions as during the establishment stage and sub-cultured every three to four weeks. For those explants which do not multiply but are healthy the BA should be increased to 2 mg/L. Those which fail to multiply on MS may do better on Anderson's medium.

**Stage III – Rooting**
The explants are transferred to MS medium without hormones or with 10µM IBA for one week and then to no hormone medium for 3 weeks. Some of the genotypes may require longer rooting periods (some blackberry cultivars can root directly in the greenhouse).

For raspberry plants activated charcoal is essential for rooting. IBA is essential for good rooting and plant growth of black and red raspberries but not for root initiation (Anderson, 1987).

**Stage IV - Hardening**
TC plants are transferred into 4" pots or 38-cell trays containing MetroMix and placed under intermittent mist for 2 wks. Plants are then maintained for 6-8 weeks under greenhouse conditions at 24-27 C and 9-14 hr photoperiod before transplanting in the field. Plants in the greenhouse are fertilized once a week with 20-20-20 liquid fertilizer.

**C. Cold Storage**
The MPUR is using the protocol developed by Reed et al. (2005) for cold storage of *Rubus* with good results. Blackberry and raspberry plantlets (2-3 cm long) are maintained in sealed tissue culture bags, one plant per section (15 x 150 mm) of a five-section bag (AgriStar Inc., Conroe, TX, USA). Each section contains 10 ml of MS or Anderson's medium without hormones, 3.5 g of Agar and 1.75 g Phytagel/L. Culture bags are maintained in the growth room for 1 week then cold acclimatized with alternating temperatures and a short photoperiod and low radiance [22 C with 8 hr photoperiod ($10\mu E \cdot m^{-2} \cdot s^{-1}$) and -1 C for 16 hours in the dark] for one week before cold storage. Plantlets are stored at 4 C and the 12 hr photoperiod 4-6 months.

The MPUR currently maintains in cold storage the following Rubus genotypes: thornless blackberry selection NC430 (35 plants); primocane fruiting blackberry selections NC533 (25), NC534 (10), NC535 (10), NC538 (25), NC539 (15); floricane fruiting red raspberry NC621 (15), Carolina (15), Pocahontas (15), and Tupy (30).

In 2008, thirty eight plants of blackberry cultivar Natchez and fortyfive plants of FF red raspberry selection NC621 (Fig.1.) were produced in the MPUR for field evaluations by NCSU breeding program.

**Conclusions:**
The MPUR has established protocols for blackberry and raspberry *in vitro* propagation and storage that have been used successfully for genotypes adapted to growing conditions in the Southeastern U.S, and advanced selections from the NCSU Bramble Breeding Program. The MPUR serves as the repository for commercial blackberry cultivars grown in the Southeastern U.S., advanced blackberry and raspberry selections from the NCSU Bramble Breeding Program and two raspberry cultivars: Mandarin and Pocahontas that are currently being evaluated for commercial production.

**Impact Statement:**
Knowledge of how to efficiently propagate blackberry and raspberry genotypes *in vitro* and in the nursery is vital to the prosperity of the bramble industry in the region. The use of micropropagated, virus-indexed and true-to-type planting stocks is expected to increase plant productivity and longevity, as well as the overall fruit quality. The MPUR at NCSU maintains a repository for bramble, strawberry and blueberry cultivars grown in the Southeastern U.S. It also serves as a source of Nuclear Stock plants for blackberry, and strawberry nurseries in North Carolina, the Southeast and the U.S.

**References:**
Anderson, W.C.1980.Tissue Culture Propagation of Red and Black Raspberries, Rubus idaeus and R. occidentalis. Acta Horticulturae. 112:13-20.

Reed, B.M., K.M. Hunter, Y. Chan and S. Gupta.2006.Medium and Long-term Storage of **Rubus** Germplasm. Acta Horticulturae. In Press.

**Fig. 1. NC621 floricane fruiting red raspberry Nuclear Stock plants in the MPUR greenhouse.**