**Cell Membrane Stability – Hot**

*Preparation*

A northern and southern plant should be paired when collecting these data. Select one leaf that is completely intact or the most intact leaf. If there are several intact leaves, select a leaf from the middle of the plant with a size large enough to punch out 20 hole-punches. If there are no leaves large enough, select two intact leaves. Wash the upper and lower surfaces of the leaf and dry on a paper towel. Use a hole punch to cut 20 leaf rounds (10 rounds from each leaf half). Place 10 hole punches into one test tube, labeled as the treatment, and 10 into a second test tube, labeled as the control. Add 10 mL of deionized water to both test tubes and cover with aluminum foil.

*Treatment*

Place the “treatment” test tube into a water bath at 55°C and the “control” test tube at room temperature for 20 minutes. After the treatment period, return test tubes to room temperature and allow to cool for 10 minutes. Measure and record the conductivity in μS for each test tube.

*Maximum Damage*

Place all test tubes in a water bath at 98°C for 60 minutes. Return all test tubes to room temperature to cool for 15 minutes. Measure and record the conductivity in μS a second time.

**Cell Membrane Stability – Cold**

*Preparation*

A northern and southern plant should be paired when collecting these data. Select one leaf that is completely intact or the most intact leaf. If there are several intact leaves, select a leaf from the middle of the plant with a size large enough to punch out 20 hole-punches. If there are no leaves large enough, select two intact leaves. Wash the upper and lower surfaces of the leaf and dry on a paper towel. Use a hole punch to cut 20 leaf rounds (10 rounds from each leaf half). Place 10 hole punches into one conical tube, labeled as the treatment, and 10 into a second conical tube, labeled as the control. Cover tubes with lids

*Treatment*

Place “treatment” conical tubes at 10°C for 24 hours, followed by 24 hours at 4°C. Transfer “treatment” conical tubes to a freezer at -18°C for 1 hour. Add 10 mL of deionized water to each conical tube and recover. Place at room temperature for 1 hour. Measure and record the conductivity in μS.

*Maximum Damage*

Place all test tubes in a water bath at 98°C for 60 minutes. Return all test tubes to room temperature to cool for 15 minutes. Measure and record the conductivity in μS a second time.