DERIVATION OF INTERSPECIFIC *SOLANUM* HYBRID GENOTYPES WITH RESISTANCE TO COLORADO POTATO BEETLE

(*Leptinotarsa decemlineata* Say)

by

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Thesis submitted to the faculty of the

Virginia Polytechnic Institute and State University

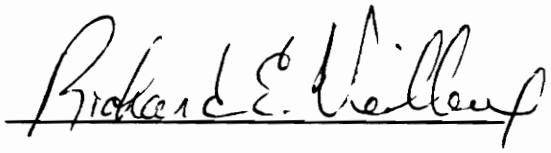
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Horticulture

APPROVED:



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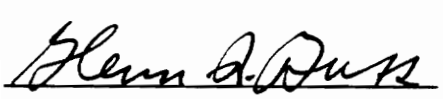

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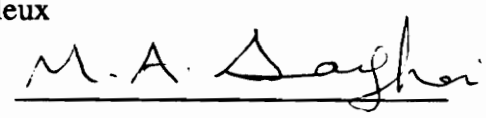

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September 1994

Blacksburg, Virginia

DERIVATION OF INTERSPECIFIC *SOLANUM* HYBRID GENOTYPES WITH RESISTANCE TO COLORADO POTATO BEETLE (*Leptinotarsa decemlineata* Say).

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(ABSTRACT)

The anther culture response of diploid *Solanum chacoense* (*chc*) - *S. phureja* (*phu*) hybrids and the regeneration potential of anther-derived monoploids was evaluated. *In vivo* evaluation of interspecific hybrids was also performed. Three hybrids were anther cultured to observe the effects of reduced nitrogen source on androgenesis. Anthers were distributed to five reduced nitrogen sources. The N concentration was 30 mM. No tested reduced nitrogen source proved superior to the control. Genotype significantly affected embryo production. Eleven monoploid genotypes were included in a leaf disc regeneration procedure utilizing three separate transfers to fresh medium differing by growth regulator composition; six genotypes responded. Silver thiosulfate (STS) at either of two steps in the process proved detrimental to diploid recovery.

Hybrids between *phu* and *chc* involving six *phu* clones and eight *chc* clones or accessions, all resistant to Colorado potato beetle (CPB), were used. No inter-family differences for germination, fruit/pollination, or seed/fruit were observed. Substantial mortality, ascribed to the phenomenon of "hybrid breakdown", occurred in three families by month four of the study. Field plantings revealed adequate CPB

resistance, while Ambush (147 g/ha) application increased total tuber weight per plant and average tuber weight. Hybrids produced less total tuber weight than *S. tuberosum* (*tbr*), while *chc* genotypes produced the smallest average tuber size. Interspecific hybrids produced tuber sizes intermediate between *chc* and *phu*. *Tbr* tubers were the largest. *Chc* families, regardless of selection for leptine glycolakaloids, suffered the least CPB damage and *phu* parental clones and hybrid families suffered the most.

ACKNOWLEDGEMENTS

Thanks to Richard Veilleux for his assistance and guidance with this project. It has been a challenge, but also a valuable learning experience.

Appreciation is also extended to Dr. Glenn R. Buss and to Dr. Mohammad A. Saghai Maroof for the assistance and insight they provided during this project and for serving on my Masters committee.

Also, thanks to Suzanne Piovano for her laboratory assistance at various stages of the 'game'.

Appreciation is due to Dr. Richard Harkess who helped to see to it that I was not committed to a psychiatric care facility during my degree process.

I would also like to thank Dr. Don Viands and Dr. Robert Mower of Cornell University, without whom I would not have been here.

Lastly, I would like to thank my parents Lennart and Elizabeth Wuosmaa for their patience and support over the years, without which I surely would not have been here.

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CHAPTER ONE: INTRODUCTION

The cultivated potato (*Solanum tuberosum* L.) has been grown for at least 8,000 years in the Andean upland regions of western South America (Rowe 1993). *Solanum* is the largest genus in the family Solanaceae, which contains between 80 and 100 genera (D'Arcy 1979, 1991). Reports on the number of species range from 1000 (D'Arcy 1979, 1991; Rowe 1993) to about 2700 (Hawkes 1963). Of the *Solanum* species, between 160 and 180 tuberize and fewer than ten are cultivated for food somewhere in the world. Among these consumed species are the pepino (*Solanum muricatum*), the eggplant (*S. melongena*) (Spooner et al. 1993), the lulu or naranjilla (*S. quitoense*), and the cocona or cubiu (*S. sessiliflorum*) (Bernardello et al. 1994), all of which are grown for their fruit. In terms of acreage planted and tonnage produced, *S. tuberosum* is the most extensively grown species worldwide, producing 250 million tons of tubers on more than 44 million acres in 125 countries. The potato is the fourth most important food crop in the world, following wheat, maize, and rice. The three most important potato producing countries in the world are: the former Soviet Union countries (25% of world production), China (19% of world production), and Poland (12% of world production). The United States accounts for less than 10% of world production, with the bulk of this concentrated in Idaho and Washington state.

The Colorado potato beetle (Coleoptera, Chrysomelidae: *Leptinotarsa decemlineata* Say) is a serious pest in most potato growing regions of the world and most potato cultivars possess very little natural resistance to the beetle. In the United States, it was present through most of the mid-west and west prior to the introduction

of the potato as a crop (Ferro and Boiteau 1993). Then, the beetle largely survived by overwintering in Mexico and migrating yearly in the spring to live on weed species, especially *Solanum elaeagnifolium* and *S. rostratum*. The shift from weedy *Solanum* hosts to *S. tuberosum* on a large scale likely required several growing seasons after the introduction of the potato into cultivation in the region (Lu and Logan 1993), actually a relatively short period in terms of insect pests. Now, the Colorado potato beetle (CPB) is distributed throughout North America and is capable of overwintering as far north as Michigan (Caprio and Grafius 1993) and New England (Weber and Ferro 1993). Adults emerge in the spring and commence feeding and laying eggs immediately. Beetle feeding is most favored by temperatures approaching 29°C, a temperature achieved during the season in most production regions (Lactin et al. 1993). A computer model has been developed to evaluate the potential dispersal patterns of CPB females, given a wide set of characters, such as population numbers, field dispersal, planting densities, and overwintering numbers (Weber et al. 1993).

USDA recommendations for pesticide control of CPB infestations may reach 12 sprayings per growing season, if infestations are quite severe. The USDA recently estimated that United States potato producers annually spend between $75 to $100 million to control CPB damage (Perlak et al. 1993). Many factors make control of CPB populations important. A CPB female can lay up to 450 eggs in her lifetime (Ferro and Boiteau 1993) and these eggs can mature into adult beetles in as little as three weeks under ideal conditions. Thus, three complete life cycles can be completed in one growing season in the warmer reaches of potato production resulting in rapidly

expanding populations. The beetle is also known to aggregate in isolated plots by use of chemical sensing, thus resulting in damage by defoliation in plots thought 'safe' or isolated from potential beetle damage (Caprio and Grafius 1993).

In recent years, the destructive potential of CPB has increased dramatically due to the development, by CPB, of synthetic chemical pesticide resistance (Forgash 1985). Pesticide resistant CPB strains are becoming so prevalent that farm test kits for detection of strains resistant to carbofuran, phosmet, esfenvalerate, and a esfenvalerate-piperonyl butoxide formulation are now available (Bishop and Grafius 1991). Computer models have been developed to investigate and predict the development of insecticide resistance. REPO is one such simulation that models derivation of pesticide adaptation given characters such as age-specific selection, temperature considerations, overwintering, population variability, migration, and many other characters (Follett et al. 1993b). Individuals in the population with resistance to an applied pesticide will be selected for and their proportion, relative to susceptible members of the population, will increase. Consequently, management of pesticide application practices must be altered to address the decreasing effectiveness of pesticides (Follett et al. 1993a). To complicate matters, commonly used and basically effective insecticides, such as organophosphate and carbamate insecticides, result in widely different levels of control among varying CPB strains that are not morphologically distinct (Wierenga and Hollingworth 1993).

To address these environmental concerns, and also to reduce synthetic chemical use, *Bacillus thuringiensis* has been extensively recommended as a biocontrol

component of integrated pest management (IPM) programs for CPB (Arpaia and Ricchiuto 1993; Ghidiu and Zehnder 1993). *B. thuringiensis* var. *tenebrionsis* and var. *san diego* are particular variants that have been shown to be effective in CPB control. The foundation of *B. t.* var. *tenebrionsis* toxicity to CPB is the production of parasporal crystalline delta endotoxin, coded by the CRYIIIA gene (Kreig et al. 1983). This protein has been shown to form cation-selective channels in lipid bi-layers which in turn cause disruption of normal membrane structure and function (Slaney et al. 1992). The CRYIIIA gene has been sequenced and inserted into the potato genome (Perlak et al. 1993). This has resulted in decreased CPB feeding behavior and reduced CPB fecundity for individuals feeding on transformed potato plants.

The truly remarkable and adaptable nature of CPB has become even more apparent to potato growers from the demonstration that CPB can develop resistance to *B. t.* var. *tenebrionsis* (Whalon et al. 1993), thus undermining efforts at transformation of the potato genome with the CRYIIIA gene. This study suggested that CPB populations may begin to exhibit resistance to *B. t.* var. *tenebrionsis* within 3-5 years in the field due to constitutive expression of the CRYIIIA protein and/or frequent applications of *B. t.* var. *tenebrionsis*. Applications of *B. t.* var. *tenebrionsis* or endotoxin formulations are not immediately lethal to adult beetles. In some cases, feeding was initially stimulated, before decreasing even on untreated foliage (Hough-Goldstein et al. 1991). This would apply strong selection pressure on CPB populations to increase tolerance or develop resistance to the CRYIIIA protein, as other CPB populations have developed tolerance and resistance to organic or inorganic

pesticides. The timing of such spraying is important, in that early spraying could increase selection pressure for resistance (Whalon et al. 1993). Current recommendations suggest spraying only at specific periods, i. e., first egg deposition or first egg hatch (Ghidiu and Zehnder 1993; Zehnder et al. 1992). Limiting spraying at these periods, it is hoped, will maximize the susceptible reaction of the beetles to the toxin, while using a minimum of spray to lengthen the period before widespread resistance occurs.

An additional means of biocontrol that has been suggested is the use of straw mulching, which reduces soil temperatures, thereby encouraging tuberization (Stoner 1993). Another benefit of straw mulch may be more favorable conditions for growth of predatory insects, such as *Coleomegilla maculata* (DeGeer) and *Lebia grandis* Henz (Riechert and Bishop 1990). In addition, straw mulch may catch dislodged larvae and prevent their climbing back onto the plant. Plastic mulching is also being suggested as a control method (Misener et al. 1993). It can prevent emergence of larvae and adults overwintering in underground plant material in the spring and entry of larvae and adults into material in the fall for overwintering. Use of fall trap crops that can be easily disrupted is another cultural control method that has been studied (Milner et al. 1992). These crops can be planted after harvest and tilled during the winter to expose overwintering adults and larvae to lethal temperature shocks.

Biocontrol utilizing the predatory stink bug (Hemiptera, Pentatomidae: *Perillus bioculatus* (F.)) has also been evaluated as a population control for CPB populations (Hough-Goldstein and Whalen 1993). *Edovum puttleri* (Hymenoptera:

Eulophidae) is an egg parasite of CPB and has been introduced into potato fields as a biocontrol agent (Mityakina et al. 1993; Ziskind and Mityakina 1991). Use of the sesquiterpene zingiberene, which is synthesized by *Lycopersicon hirsutum* f. *hirsutum* and *Lycopersicon hirsutum* PI 126445 is a further biocontrol input (Rahimi and Carter 1993). This chemical may be used as a spray or the gene may be engineered into the *S. tuberosum* genome, but overapplication may result in similar problems that have been encountered with other insecticides and may be encountered with *Bacillus thuringiensis*. The use of other 'non-traditional' pesticide chemicals to control CPB feeding has been investigated. These include the citrus limonoids and limonin derivatives limonin, nomilin, obacunonoe, epilimonol, and limonin diosphenol and the corresponding salts of all these compounds (Liu et al. 1991). This group of compounds is useful in that these materials are absorbed by foliar tissue and become systemic after application without being phytotoxic. The salts themselves are not effective but are converted into the free molecule after incorporation by the plant, such as limonin salt converted into limonin. These compounds essentially only differ in the structure of their A rings (Liu et al. 1991; Mendel et al. 1991). This is important for selecting the appropriate chemical for resistance to some insects, but the variation of A ring structure has been shown to be inconsequential for CPB control (Mendel et al. 1991).

Introgression of genes from wild *Solanum* species, such as *Solanum chacoense* Bitt. (Costa and Gaugler 1989) and *S. berthaultii* Hawkes (Neal et al. 1991; Pelletier and Smilowitz 1990; Plaisted et al. 1992) has been proposed as an alternative for, or a

complement to, pesticide, *Bacillus thuringiensis*, cultural, and predatory or antagonistic insect control of CPB. *S. berthaultii* (2n=2x=24) exhibits resistance to various insects, including CPB, by means of secretion of a sticky exudate from its foliar Type B trichome (Pelletier and Smilowitz 1990). This exudate, alone or with exudates from the Type A trichomes, traps insects so that they may not move, which results in eventual death by starvation. Larval stages or smaller insects are also killed by the trichomes as a result of slowed movement and starvation resulting from the physical barrier presented by the trichomes. Adult females are also particularly sensitive to the foliage. Some clones are also particularly undigestible to CPB individuals and therefore provide little nutrition (Franca and Tingey 1994). This can cause disrupted digestion and damage or death to ovaries by lack of nutrition or by pressure exerted on the ovaries by an overfilled gut of undigested foliar material. *S. chacoense* (2n=2x=24) is one of several *Solanum* species that has been reported resistant to Colorado potato beetle (Carter 1987), predominantly by the production of alkaloids that have been related to insect resistance (Sinden et al. 1986). This species, one of the most diverse of the *Solanum* section *Tuberarium* (Correll 1962), has a wide native habitat in South America. It is nearly endemic in southern Bolivia, Paraguay, Uruguay, northern Argentina, and southern Brazil. It occurs commonly in disturbed fields, forest edges, and in rangelands. In addition, it mostly grows below 3200 meters elevation, in habitats normally too warm for most tuber-bearing *Solanum* species.

Acetylated glycoalkaloids (leptines) are the primary alkaloids that confer CPB resistance in *Solanum chacoense* (Sinden et al. 1986) and are among several unusual alkaloids produced by the species (Osman et al. 1976). Most notable among the leptine glycoalkaloids is acetylleptinidine. This chemical is toxic to all stages of CPB development (Melville et al. 1985). Larval feeding has resulted in delayed development or death while adult feeding has resulted in sterility and/or death. Leptine glycoalkaloids can also act as deterrents to feeding if used in a spray application (Costa and Gaugler 1989) by interacting with galeal chemosensory cells (Mitchell 1987).

There are advantages and disadvantages to the use of *Solanum chacoense* in potato breeding programs as have been shown in previous breeding projects. The main advantages are its heat tolerance (Reynolds and Ewing 1989) and glycoalkaloid synthesis pathways (Sinden et al. 1986). The primary benefit of this particular glycoalkaloid synthesis pathway is the localized expression of its end-products within the above-ground portions of the plant. Studies have shown (Sanford et al. 1992) that the development of *S. tuberosum* genotypes with glycoalkaloid producing *S. chacoense* clones in their pedigree will not generate glycoalkaloid levels above those deemed safe in tuber tissue while foliar concentrations are sufficiently high to control potato leafhopper infestations. In addition, increased heat tolerance could expand potato production to warmer regions of Africa and Asia (Ewing 1981; Nowak and Colborne 1989). People in developing countries may benefit from increased production of

potato, a crop that could increase protein, carbohydrate, vitamin, and mineral content in their diets.

S. chacoense is somewhat limited in its usefulness, however, in that it is an endemic weed in much of its native range of South America. Due to potential strong linkages between undesirable growth habits and its favorable traits, it is probable that there will be difficulty in the transferral of heat tolerant and glycoalkaloid genes from the *S. chacoense* genome during the breeding process. The unfavorable traits that are most notable include production of large numbers of small tubers which form on stolons that may be meters long. Small tuber size may present a problem to extensive use of *S. chacoense* in that it has been shown that prebreeding of wild potato species for increased tuberization is not extraordinarily beneficial in the F_1 generation of wild species-*S. tuberosum* hybrids (Jacobsen and Jansky 1989). Furthermore, leptine glycoalkaloid biosynthesis is not uniformly high in all accessions of *S. chacoense*. In the rare high glycoalkaloid producing accessions, few representatives produce high enough levels of leptines to be useful (Sinden et al. 1986).

A breeding strategy to capture the desirable traits of *Solanum chacoense* in a more adapted form is the hybridization of *S. chacoense* (2n=2x=24) with *S. phureja* (2n=2x=24) to generate interspecific F_1 hybrids (Bani-Aameur et al. 1991, 1993). These hybrids can then be selected for vigor, CPB resistance, and the presence of 2n gametes, with eventual hybridization with *S. tuberosum* (2n=4x=48) to generate mostly vigorous 4x-2x hybrids (Arndt and Peloquin 1990; Bani-Aameur et al. 1991; Schroeder and Peloquin 1983; Stelly and Peloquin 1985; Werner and Peloquin 1991;

Yerk and Peloquin 1990). *S. phureja* is more adapted, in terms of growth habit and tuber production than *S. chacoense* and is more closely related to *S. tuberosum* (Owen et al. 1988a). The 'species' *S. phureja* refers to an assemblage of variable diploid genotypes native to Venezuela, Colombia, Ecuador, and northern Peru that do not possess tuber dormancy (Correll 1962). F_1 hybrids between various *S. chacoense* and *S. phureja* clones or accessions have been generated (Bani-Aameur et al. 1991; Grun and Chu 1978). Difficulty in producing successful hybrids that are sufficiently fertile and vigorous in growth habit to be adequately utilized in breeding programs has occasionally been reported (Bani-Aameur et al. 1991). Some variability for hybrid vigor is to be expected, considering the variability of the parental materials and their taxonomic divergence. However, the extent and type of variation have been more extreme than expected given the vigor of the parental material. The range of variation has included all the following results of hybridization: no hybrids due to poor or no seed set; no hybrids due to no germination of F_1 seed; germination of F_1 seed, but unhealthy seedlings that die shortly after germination; germination and some growth followed by unexpected decline; germination and some growth, followed by decline and regrowth; germination and extremely vigorous growth, but sexual sterility; and germination and extremely vigorous growth with fertility exceeding either parent. In addition, it is interesting that some of the vigorous and fertile hybrids will only grow vigorously and flower in the extreme heat of summer, either in the greenhouse or field, conditions detrimental to most other *Solanum* species and cultivars, including

both parents of the hybrids. This would appear to be heat tolerance, or perhaps a heat requirement, inherited from *S. chacoense*.

In *Solanum*, many interspecific hybrids have been generated. The most directly useful to breeding programs involve crosses with *S. tuberosum*. Some examples include: 1) 2x-2x crosses between *S. tuberosum* X *S. chacoense* (Cardi et al. 1993; Conicella et al. 1991; Douches and Quiros 1988; Hermundstad and Peloquin 1985; Sanford and Ladd 1992), *S. commersonii* X *S. tuberosum* (Cardi et al. 1993), *S. tuberosum* X *S. kurtizianum*, *S. tuberosum* X *S. spegazzinii* (2n=24), *S. tuberosum* X *S. tarijense* (Hermundstad and Peloquin 1985), *S. tuberosum* X *S. brevidens* (Jacobsen et al. 1993), *S. tuberosum* X *S. berthaultii* (Hermundstad and Peloquin 1985; Kalazich and Plaisted 1991; Mehlenbacher et al. 1983; Neal et al. 1991; Pelletier and Smilowitz 1990; Plaisted et al. 1992), 2x (*S. phureja* X *S. tuberosum*) X *S. berthaultii* (Mehlenbacher et al. 1983), *S. tuberosum* X *S. chancoyense*, *S. tuberosum* X *S. commersonii*, *S. tuberosum* X *S. cardiophyllum*, *S. tuberosum* X *S. jamesii*, *S. tuberosum* X *S. machicense*, *S. tuberosum* X *S. pinnatisectum*, and *S. tuberosum* X *S. trifidum* (Novy and Hanneman 1991); 2) 4x-2x crosses between *S. tuberosum* X (*S. phureja* X *S. stenotomum*) (Abdul-baki and Haynes 1993), *S. tuberosum* X *S. chacoense* (Conicella et al. 1991; Douches and Quiros 1988; Sanford and Ladd 1992), *S. tuberosum* X *S. phureja* (Clulow et al. 1991), *S. tuberosum* X S. bukasovii , *S. tuberosum* X *S. gourlayi*, *S. tuberosum* X *S. multidissectum*, *S. tuberosum* X *S. vernei*, *S. tuberosum* X *S. verrucosum* (Yerk and Peloquin 1990), and *S. stoloniferum* X 2x (*S. tuberosum* X *S. phureja*) (Brown 1988); 3) 4x-4x crosses between *S. stoloniferum* X *S.*

tuberosum (Brown and Adiwilaga 1991) and *S. acaule* X *S. tuberosum* (Camadro and Espinillo 1990); and 4) the 6x-4x cross *S. hougasii* X *S. tuberosum* (Brown et al. 1991). Other more distantly useful crosses include: *S. stoloniferum* (2n=2x=48) X (*S. phureja* [2n=24] X *S. phureja* [2n=24]) (Brown 1988), *S. sparsipilum* (2n=24) X *S. stenotomum* (2n=24) (Rabinowitz et al. 1990), *S. chacoense* (2n=24) X *S. berthaultii* (2n=24) and *S. chacoense* (2n=24) X *S. tarijense* (2n=24) (Mooney and Jansky 1990), 6x (*S. acaule* [2n=48] X *S. bulbocastanum* [2n=24]) X *S. phureja* (2n=24) (Ramanna and Hermsen 1971), *S. polytrichon* (2n=48) X *S. phureja* (2n=24) (Abdalla and Ramanna 1971), *S. phureja* (2n=24) X *S. stenotomum* (2n=24) (Vallejo et al. 1994a, 1994b), and *S. chacoense* (2n=24) X *S. commersonii* (2n=24). Certain of these hybrids have been shown to be somewhat unstable or unpredictable (Summers and Grun 1981).

There are many potential mechanisms for the hybrid breakdown phenomenon observed in some *Solanum chacoense* X *S. phureja* F_1 hybrids. Accumulation of seed set genes within the genomes of the two species has been suggested, as has been hypothesized for the F_1 and F_2 hybrid populations of *Solanum chacoense* X *S. commersonii* (Summers and Grun 1981). These sets of genes interfere with each other within hybrid genomes of the two species to limit maturation of viable seed, therefore establishing reproductive barriers. Use of 'Mexican' *Solanum* species as females in generation of F_1 hybrids with *Solanum* species native to South America has also been suggested as a potential cause of sterility. Such families exhibit differing vigor in reciprocal crosses. This suggests that Mexican native cytoplasms are sterility inducing

in Mexican-South American F_1 hybrids, as has been hypothesized for *S. polytrichon* X *S. phureja* hybrids (Abdalla and Ramanna 1971). A third potential source of interspecific hybrid difficulty is somatic chromosomal elimination at early developmental stages, as observed for the three-way hybrid 6x (*S. acaule* X *S. bulbocastanum*) X 2x *S. phureja* (Ramanna and Hermsen 1971). Further problems have been involved when hybrid parents with varying endosperm balance numbers (EBN) are crossed (Novy and Hanneman 1991). Each species is assigned a value, depending on its endosperm ploidy, and species with the same nuclear ploidy may possess different EBN number. If crosses between species with differing EBN numbers are made, seeds may not develop normally, due to endosperm abnormalities, as is the case for hybrids between 4x *S. stoloniferum* X 2x (2x *S. tuberosum* X 2x *S. phureja*) (Brown 1988) and 4x *S. stoloniferum* X 4x *S. tuberosum* (Brown and Adiwilaga 1991).

Such hybrid breakdown, or dysgenesis, has also been noted in several other genera, including: *Cucurbita* (Cutler and Whitaker 1969; Weeden and Robinson 1986), *Gossypium* (Harland 1936; Stephens 1949, 1950), *Platanus* (Sax 1933), *Campsis* (Sax 1933), and others (Grant 1966; Stebbins 1945). Such F_1 hybrid instability has been attributed most often to gross differences in chromosomal structure between the component species of the hybrid (Sax 1933; Weeden and Robinson 1986). This incompatibility in chromosome structure results in disruption of cellular division and concomitant death of the hybrid at a stage prior to sexual reproduction. Other explanations cited for the phenomenon are chromosomal elimination (Harland 1936;

Stephens 1949), gene substitution and cryptic differentiation (Stephens 1950), and other types of hybrid genetic destabilization (Grant 1966).

A potential method for selection of useful recombined genotypes from vigorous interspecific *Solanum* hybrids may be to utilize the tissue culture process of anther culture to derive monoploids, some of which may be more vigorous than their donor parents. Anther culture of potato has received much attention and a relatively reliable system has been developed for production of androgenic embryos (Powell and Uhrig 1987; Uhrig 1985; Wenzel and Uhrig 1981). Response of a clone to anther culture can be highly variable, even from homozygous donor genotypes (Snape et al. 1988; Snider and Veilleux 1994). In addition, ploidy level of androgenic material has been variable. Flow cytometric analysis of plant materials, including anther derived plants, has been shown to be an efficient and rapid means of determining ploidy with small amounts of plant material (Bergounioux et al. 1986; Bharathan et al. 1994; Birhman et al. 1994; Ho and Rayburn 1991; Owen et al. 1988b; Ozias-Akins and Jarret 1994; Sharma et al. 1983; ten Cate and Ramulu 1987; Waara et al. 1991), especially when compared to methods such as root tip squashes or guard cell chloroplast density determination (Singsit and Veilleux 1991).

Monoploid potato clones, after proceeding through the so-called 'monoploid sieve' (Wenzel et al. 1979) to eliminate deleterious gene combinations, could be further selected for traits of interest, such as vigor, heat tolerance, or leptine glycoalkaloid synthesis. This material would serve as a source of doubled monoploids (2n=24) (Mollers et al. 1992) or protoplasts to be fused with other favorable

monoploid genotypes (Wenzel et al. 1979). Protoplast fusion of widely differing genotypes offers the potential to produce highly heterozygous materials not generable by more conventional means (Knopf and Bromova 1987; Mollers and Wenzel 1992; Thach et al. 1993; Waara et al. 1991) or to engage in genetic studies (Ozminkowski and Jourdan 1994a, 1994b). Novel germplasm development in *Solanum* by means of protoplast fusion is exemplified by the production of *S. commersonii* X *S. tuberosum* hybrids (Cardi et al. 1993), *S. tuberosum* X *S. brevidens* hybrids (Jacobsen et al. 1993), and hybrids of *S. tuberosum* and *Lycopersicon esculentum* (Jacobsen et al. 1992). These are all hybrids that would not normally be possible due to sexual barriers. These methods could serve as potent breeding tools for introgression of wild germplasm into the *S. tuberosum* genome.

Objectives

Introgression of wild gene combinations into the *Solanum tuberosum* genome for the purposes of increased vigor, insect resistance, and heat tolerance assumes that the wild genotypes and interspecific hybrids are sufficiently fertile to make sexual crossing possible or sufficiently amenable to tissue culture for protoplast manipulation. To address the issues of hybridization and utility of hybrids, hybrids were generated, studied, and selected. Following are the overall research objectives addressed in this thesis:

- ⇨ Generation and observation of stability, vigor, and fertility of *Solanum chacoense* x *S. phureja* and *S. phureja* x *S. chacoense* hybrids to evaluate the feasibility of utilizing such hybrids for introgression of desirable genes into the *S. tuberosum* genome.
- ⇨ Evaluation of existing interspecific *Solanum chacoense* x *S. phureja* and *S. phureja* x *S. chacoense* genotypes and their androgenic derivatives for genetic stability.
- ⇨ Observation of androgenic embryo production and ploidy response of *S. chacoense* x *S. phureja* and *S. chacoense* x *S. phureja* interspecific hybrids to varying reduced nitrogen sources in anther culture (Kamada and Harada 1984a, 1984b; Wetherell and Dougall 1976).

⇨ Observation of the reaction of resulting monoploids to the presence or absence of silver thiosulfate (STS) in various steps of the leaf disc regeneration process (Mollers et al. 1992; Perl et al. 1988).

Literature Cited

Abdalla MF and Ramanna MS (1971) Male sterility in *Solanum polytrichon* X *S. phureja* hybrid, caused by plasmon-genic interaction and its significance. Euphytica 20: 482-489.

Abdul-Baki AA and Haynes KG (1993) Male fertility of derived tetraploids of *Solanum tuberosum* from groups Tuberosum X Phureja-Stenotomum. Amer. Potato J. 70: 885-895.

Arndt GC and Peloquin SJ (1990) The identification and evaluation of hybrid plants among open pollinated true seed families. Amer. Potato J. 67: 393-404.

Arpaia S and Ricchiuto B (1993) Effects of *Bacillus thuringiensis* toxin extracts on feeding behavior of Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 22: 334-338.

Bani-Aameur F, Lauer FI, and Veilleux RE (1993) Enhancement of diploid *Solanum chacoense* Bitt. using adapted clones of *Solanum phureja* Juz. & Buk. Euphytica 68: 169-179.

Bani-Aameur F, Lauer FI, Veilleux RE, and Hilali A (1991) Genomic composition of 4x-2x potato hybrids: influence of *Solanum chacoense*. Genome 34: 413-420.

Bergounioux C, Perennes C, Miege C, and Gadal P (1986) The effect of male sterility on protoplast division in *Petunia hybrida*. Cell cycle comparison by flow cytometry. Protoplasma 130: 138-144.

Bernardello LM, Heiser CB, and Piazzano M (1994) Karyotypic studies in *Solanum* section *Lasiocarpa* (Solanaceae). Amer. J. Bot. 81: 95-103.

Bharathan G, Lambert G, and Galbraith DW (1994) Nuclear DNA content of monocotyledons and related taxa. Amer. J. Bot. 81: 381-386.

Birhman RK, Rivard SR, and Cappadocia M (1994) Restriction fragment length polymorphism analysis of anther-culture-derived *Solanum chacoense*. HortScience 29: 206-208.

Bishop BA and Grafius E (1991) An on-farm insecticide resistance test kit for Colorado potato beetle (Coleoptera: Chrysomelidae). Amer. Potato J. 68: 53-64.

Brown CR (1988) Characteristics of 2N pollen producing triploid hybrids between *Solanum stoloniferum* and cultivated diploid potatoes. Amer. Potato J. 65: 75-84.

Brown CR and Adiwilaga KD (1991) Use of rescue pollination to make a complex interspecific cross in potato. Amer. Potato J. 68: 813-820.

Brown CR, Mojtahedi H, and Santo GS (1991) Resistance to Columbia root-knot nematode in *Solanum* spp. and in hybrids of *S. hougasii* with tetraploid cultivated potato. Amer. Potato J. 68: 445-452.

Camadro EL and Espinillo JC (1990) Germplasm transfer from the wild tetraploid species *Solanum acaule* Bitt. to the cultivated potato, *S. tuberosum* L. using 2N eggs. Amer Potato J. 67: 737-749.

Caprio MA and Grafius EJ (1993) Movement of adult Colorado potato beetles, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), in response to isolated potato plots. Great Lakes Entomol. 26: 223-231.

Cardi T, Ambrosio FD, Consoli D, Puite KJ, and Ramulu KS (1993) Production of somatic hybrids between frost-tolerant *Solanum commersonii* and *S. tuberosum*: characterization of hybrid plants. Theor. Appl. Genet. 87: 193-200.

Carter CD (1987) Screening *Solanum* germplasm for resistance to Colorado potato beetle. Amer. Potato J. 64: 563-568.

Clulow SA, Wilkinson MJ, Waugh R, Baird E, DeMaine MJ, and Powell W (1991) Cytological and molecular observations on *Solanum phureja*-induced dihaploid potatoes. Theor. Appl. Genet. 82: 545-551.

Conicella C, Barone A, Del Giudice A, Frusciante L, and Monti LM (1991) Cytological evidences of SDR-FDR mixture in the formation of 2n eggs in a potato diploid clone. Theor. Appl. Genet. 81: 59-63

Correll DS (1962) The Potato and its Wild Relatives. Texas Research Foundation. Renner, Texas. 606 pp.

Costa SD and Gaugler R (1989) Influence of *Solanum* host plants on Colorado potato beetle (Coleoptera: Chrysomelidae) susceptibility to the entomopathogen *Beauveria bassiana*. Environ. Entomol. 18: 531-536.

Cutler HC and Whitaker TW (1969) A new species of *Cucurbita* from Ecuador. Ann. Missouri Bot. Gard. 55: 392-396.

D'Arcy WG (1991) The Solanaceae since 1976, with a review of its biogeography. In: Hawkes JG Lester RN, Nee M, and Estrada N (Eds) Solanaceae III: taxonomy, chemistry, evolution. Royal Botanic Gardens. Kew, England. pp. 75-137.

D'Arcy WG (1979) The classification of the Solanaceae. In: Hawkes JG, Lester RN, and Skelding AD (Eds.) The biology and taxonomy of the Solanaceae. Academic Press. London, England. pp. 3-47.

Douches DS and Quiros CF (1988) Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* X *S. chacoense* diploid hybrid. Heredity 60: 183-191.

Ewing EE (1981) Heat stress and the tuberization response. Amer. Potato J. 58: 31-49.

Ferro DN and Boiteau G (1993) Management of insect pests. In: Rowe RC (Ed.) (1993) Potato Health Management. APS Press. St. Paul. pp. 103-108.

Follett PA, Gould F, and Kennedy GG (1993a) Comparative fitness of three strains of Colorado potato beetle (Coleoptera: Chrysomelidae) in the field: spatial and temporal variation in insecticide selection. J. Econ. Entomol. 86: 1324-1333.

Follett PA, Kennedy GG, and Gould F (1993b) REPO: a simulation model that explores Colorado potato beetle (Coleoptera: Chrysomelidae) adaptation to insecticides. Environ. Entomol. 22: 283-296.

Forgash AJ (1985) Insecticide resistance in the Colorado potato beetle. In: Ferro DN and Voss RH Eds. Proceedings of the Symposium on the Colorado potato beetle, 17th Congress of Entomology. pp. 33-52.

Franca FH and Tingey WM (1994) *Solanum berthaultii* Hawkes affects the digestive system, fat body and ovaries of the Colorado potato beetle. Amer. Potato J. 71: 405-410.

Ghidiu GM and Zehnder GW (1993) Timing of the initial spray application of *Bacillus thuringiensis* for control of the Colorado potato beetle (Coleoptera: Chrysomelidae) in potatoes. Biol. Cont. 3: 348-352.

Grant V (1966) Block inheritance of viability genes in plant species. Amer. Nat. 100: 591-601.

Grun P and Chu L (1978) Development of plants from protoplasts of *Solanum* (Solanaceae). Amer. J. Bot. 65: 538-544.

Harland SC (1936) The genetical conception of a species. Biol. Rev. 11: 83-112.

Hawkes JG (1963) A revision of the tuber-bearing Solanums, 2nd ed. Scottish Plant Breeding Station Record 1963. Pentlandfield, Scotland. pp. 76-181.

Hermundstad SA and Peloquin SJ (1985) Male fertility and 2N pollen production in haploid-wild species hybrids. Amer. Potato J. 62: 479-487.

Ho I and Rayburn A (1991) The relationship between chloroplast number and genome size in *Zea mays* var. *mays*. Plant Sci. 74: 255-260.

Hough-Goldstein J and Whalen J (1993) Inundative release of predatory stink bugs for control of Colorado potato beetle. Biol. Cont. 3: 343-347.

Hough-Goldstein J, Tisler AM, Zehnder GW, and Uyeda KA (1991) Colorado potato beetle (Coleoptera: Chrysomelidae) consumption of foliage treated with *Bacillus thuringiensis* var. *san diego* and various feeding stimulants. J. Econ. Entomol. 84: 87-93.

Jacobsen E, Malvar R, Hulgen DJ, Bergervoet JEM, and Ramanna MS (1993) Isolation and characterisation of somatic hybrids of diploid *Solanum tuberosum* and *Solanum brevidens* and the use of amylose-free starch mutation for detection of introgression. Euphytica 69: 191-201.

Jacobsen E, Reinhout P, Bergervoet JEM, de Looff J, Abidin PE, Huigen DJ, and Ramanna MS (1992) Isolation and characterization of potato-tomato somatic hybrids using an amylose-free potato mutant as parental material. Theor. Appl. Genet. 85: 159-164.

Jacobsen TL and Jansky SH (1989) Effects of pre-breeding wild species on tuberization of *Solanum tuberosum* haploid-wild species hybrids. Amer. Potato J. 66: 803-811.

Kalazich JC and Plaisted RL (1991) Association between trichome characters and agronomic traits in *Solanum tuberosum* (L.) X *S. berthaultii* (Hawkes) hybrids. Amer. Potato J. 68: 833-847.

Kamada H and Harada H (1984a) Studies on nitrogen metabolism during somatic embryogenesis in carrot. I. Utilization of α-alanine as a nitrogen source. Plant Sci. Lett. 33: 7-13.

Kamada H and Harada H (1984b) Changes in endogenous amino acid composition during somatic embryogenesis in *Daucus carota* L. Plant & Cell Physiol. 25: 27-38.

Knopf UC and Bromova M (1987) Electronic selection and sorting of leaf protoplasts from *Nicotiana tabacum* (var. Wisconsin) by autofluorescence using a closed sorting device and regeneration of entire plants. Eur. J. Cell Biol. 44: 328-332.

Kreig A, Huger AM, Langenbruch GA, and Schnetter W (1983) *Bacillus thuringiensis* var. *tenebrionis*; ein neuer gegenuber Larven von coleopteren wirksamer Pathotyp. Z. Agnew. Entomol. 96: 500-508.

Lactin DJ, Holliday NJ, and Lamari LL (1003) Temperature dependence and constant-temperature diel aperiodicy of feeding by Colorado potato beetle larvae (Coleoptera: Chrysomelidae) in short-duration laboratory studies. Environ. Entomol. 22: 784-790.

Liu Y, Alford AR, and Bentley MD (1991) Changes in antifeedant activity of limonin double salt in potato leaves. J. Econ. Entomol. 84: 1154-1157.

Lu W and Logan P (1993) Induction of feeding on potato in Mexican *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Environ. Entomol. 22: 759-765.

Mehlenbacher SA, Plaisted RL, and Tingey WM (1983) Inheritance of glandular trichomes in crosses with *Solanum berthaultii*. Amer. Potato J. 60: 699-708.

Melville AA, Storch RH, Bushway RJ, and Alford AR (1985) Growth and feeding of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera Chrysomelidae), fed foliage of three *Solanum* species. Maine Ag. Exp. St. Tech. Bull. 115.

Mendel MJ, Alford AR. Rajab MS, and Bentley MD (1991) Antifeedant effects of citrus limonoids differing in A-ring structure on Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 84: 1158-1162.

Milner M, Kung KS, Wyman JA, Feldman J, and Nordheim E (1992) Enhancing overwintering mortality of Colorado potato beetle (Coleoptera: Chrysomelidae) by manipulating the temperature of its diapause habitat. J. Econ. Entomol. 85: 1701-1708.

Misener GC, Boiteau G, and McMillan LP (1993) Amer. Potato J. A plastic-lining trenching device for the control of Colorado potato beetle: Beetle Excluder. Amer. Potato J. 70: 903-908.

Mitchell BK (1987) Interactions of alkaloids with galeal chemosensory cells of Colorado potato beetle. J. Chem. Ecol. 13: 2009-2022.

Mityakina ON, Ziskind LA, Izhavski SS, and Klimenko A (1993) The effects of temperature and humidity on the development of *Edovum puttleri* (Hymenoptera, Eulophidae), an egg parasite of Colorado potato beetle. Zoologichensky Zuhrnal 72: 118-124.

Mollers C and Wenzel G (1992) Somatic hybridization of dihaploid potato protoplasts as a tool for potato breeding. Bot. Acta 105: 133-139.

Mollers C, Zhang S, and Wenzel G (1992) The influence of silver thiosulfate on potato protoplast cultures. Plant Breeding 108: 12-18.

Mooney JJ and Jansky SH (1990) Development of insect resistant interspecific *Solanum* hybrids. Proc. N. D. Acad. Sci. 44: 73. (Abstr.)

Neal JJ, Plaisted RL, and Tingey WM (1991) Feeding behavior and survival of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larvae on *Solanum berthaultii* Hawkes and an F_6 *S. tuberosum* L. X *S. berthaultii* hybrid. Amer. Potato J. 68: 649-658.

Nova RG and Hanneman Jr. RE (1991) Hybridization between gp. tuberosum haploids and 1EBN wild potato species. Amer. Potato J. 68: 151-169.

Nowak J and Colborne D (1989) *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. Amer. Potato J. 66: 35-45.

Osman SF, Herb SF, Fitzpatrick TJ, and Sinden SL (1976) Commersonine. A new alkaloid from two *Solanum* species. Phytochemistry 15: 1065-1067.

Owen HR, Veilleux RE, Haynes FL, and Haynes KG (1988a) Photoperiod effects on 2N pollen production, response to anther culture, and net photosynthesis of diplandrous clone of *Solanum phureja*. Amer. Potato J. 65: 131-139.

Owen HR, Veilleux RE, Levy D, and Ochs DL (1988b) Environmental, genotypic, and ploidy effects on endopolyploidization within a genotype of *Solanum phureja* and its derivatives. Genome 30: 506-510.

Ozias-Akins P and Jarret RL (1994) Nuclear DNA content and ploidy levels in the genus *Ipomoea*. J. Amer. Soc. Hort. Sci. 119: 110-115.

Ozminkowski Jr. RH and Jourdan P (1994A) Comparing the resynthesis of *Brassica napus* L. by interspecific somatic and sexual hybridization. I. Producing and identifying hybrids. J. Amer. Soc. Hort. Sci. 119: 808-815.

Ozminkowski Jr. RH and Jourdan P (1994B) Comparing the resynthesis of *Brassica napus* L. by interspecific somatic and sexual hybridization. II. Hybrid morphology and identifying organelle genomes. J. Amer. Soc. Hort Sci. 119: 816-823.

Pelletier Y and Smilowitz Z (1990) Effect of trichome B exudate of *Solanum berthaultii* Hawkes on consumption by the Colorado potato beetle, *Leptinotarsa decemlineata*. J. Chem. Ecol. 16: 1547-1555.

Perl A, Aviv D, and Galun E (1988) Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. Plant Cell Rep. 7: 403-406.

Perlak FJ, Stone TB, Muskopf YM, Petersen LJ, Parker GB, McPherson SA, Wyman J, Love S, Reed G, Biever D, and Fischoff DA (1993) Genetically improved potatoes: protection from damage by Colorado potato beetle. Plant Mol. Biol. 22: 313-321.

Plaisted RL, Tingey WM, and Steffens JC (1992) The germplasm release of NYL 235-4, a clone with resistance to the Colorado potato beetle. Amer. Potato J. 69: 843-846.

Powell W and Uhrig H (1987) Anther culture of *Solanum* genotypes. Plant Cell Tiss. Organ Cult. 11: 13-24.

Rabinowitz D, Linder CR, Ortega R, Begazo D, Murguia H, Douches DS, and Quiros CF (1990) High levels of interspecific hybridization *Solanum sparsipilum* and *S. stenotomum* in experimental plots in the Andes. Amer. Potato J. 67: 71-81.

Rahimi FR and Carter CD (1993) Inheritance of zingiberene in *Lycopersicon*. Theor. Appl. Genet. 87: 593-597.

Ramanna MS and Hermsen JG (1971) Somatic chromosome elimination and meiotic chromosome pairing in the triple hybrid 6x-(*Solanum acaule* X *S. bulbocastanum*) X 2x-*S. phureja*. Euphytica 20: 470-481.

Reynolds MP and Ewing EE (1989) Heat tolerance in tuber bearing *Solanum* species: a protocol for screening. Amer. Potato J. 66: 63-73.

Riechert SE and Bishop L (1990) Prey control by an assemblage of generalist predators: spiders in the garden test systems. Ecology 71: 1441-1450.

Rowe RC (1993) Potato health management: a holistic approach. In: Rowe RC (Ed.) (1993) Potato Health Management. APS Press. St. Paul. pp. 1-10.

Sanford LL, Deahl KL, Sinden SL, and Ladd Jr. TL (1992) Glycoalkaloid content in tubers from *Solanum tuberosum* populations selected for potato leafhopper resistance. Amer. Potato J. 69: 693-703.

Sanford LL and Ladd Jr. TL (1992) Performance of populations derived by selecting for resistance to potato leafhopper in a 4x *Solanum tuberosum* X 2x *Solanum chacoense* cross. Amer. Potato J. 69: 391-400.

Sax K (1933) Species hybrids in *Platanus* and *Campsis*. J. Arnold Arbor. 14: 274-278.

Schroeder SH and Peloquin SJ (1983) Seed set in 4x x 2x crosses as related to 2N pollen frequency. Amer. Potato J. 60: 527-536.

Sharma E, Firoozabady E, Ayres NW, and Galbraith DW (1983) Improvement of anther culture in *Nicotiana*: media, culture conditions and flow cytometric determination of ploidy level. Z. Pflanzenphysiol. 111: 441-451.

Sinden SL, Sanford LL, Cantelo WW, and Deahl KL (1986) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in *Solanum chacoense*. Environ. Entomol. 15: 1057-1062.

Singsit C and Veilleux RE (1991) Chloroplast density in guard cells of leaves of anther-derived potato plants grown *in vitro* and *in vivo*. HortScience 26: 592-594.

Slaney AC, Robbins HL, and English L (1992) Mode of action of *Bacillus thuringiensis* toxin CRYIIIA: an analysis of toxicity in *Leptinotarsa decemlineata* (Say) and *Diabrotica undecimpunctata howardi* Barber. Insect Biochem. Mol. Biol. 22: 9-18.

Snape JW, Sitch LA, Simpson E, and Parker BB (1988) Tests for the presence of gametoclonal variation in barley and wheat doubled haploids produced using the *Hordeum bulbosum* system. Theor. Appl. Genet. 75: 509-513.

Snider KT and Veilleux RE (1994) Factors affecting variability in anther culture and in conversion of androgenic embryos of *Solanum phureja*. Plant Cell Tiss. Organ Cult. 36: 345-354.

Spooner DM, Anderson GJ, and Jansen RK (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Amer. J. Bot. 80: 676-688.

Stebbins Jr. GL (1945) The cytological analysis of species hybrids. II. Bot. Rev. 11: 463-486.

Stelly DM and Peloquin SJ (1985) Screening for 2n female gametophytes, female fertility, and 4x x 2x crossability in potatoes (*Solanum* spp.). Amer. Potato J. 62: 519-529.

Stephens SG (1950) The internal mechanism of speciation in *Gossypium*. Bot. Rev. 16: 115-149.

Stephens SG (1949) The cytogenetics of speciation in *Gossypium*. I Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics 34: 627-637.

Stoner AK (1993) Effects of straw and leaf mulches and trickle irrigation on the abundance of Colorado potato beetles (Coleoptera: Chrysomelidae) on potato in Connecticut. J. Entomol. Sci. 28: 393-403.

Summers D and Grun P (1981) Reproductive isolation barriers to gene exchange between *Solanum chacoense* and *S. commersonii* (Solanaceae). Amer. J. Bot. 68: 1240-1248.

Ten Cate CHH and Ramulu KS (1987) Callus growth, tumor development and polyploidization in the tetraploid cultivar Bintje. Plant Sci. 49: 209-216.

Thach NW, Frei U, and Wenzel G (1993) Somatic fusion for combining virus resistances in *Solanum tuberosum* L. Theor. Appl. Genet. 85: 863-867.

Uhrig H (1985) Genetic selection and liquid medium conditions improve the yield of androgenetic plants from diploid potatoes. Theor. Appl. Genet. 71: 455-460

Vallejo RL, Collins WW, and Moll, RH (1994a) Inheritence of A and B trichome density and polyphenol oxidase activity in diploid potatoes. J. Amer. Soc. Hort. Sci. 119: 829-832.

Vallejo RL, Collins WW, and Schiavone RD (1994b) Genetics and incorporation of glandular trichomes and polyphenol oxidase activity into an advanced *Solanum phureja-S. stenotomum* diploid potato population. J. Amer. Soc. 0Hort. Sci. 119: 824-828

Waara S, Wallin A, and Eriksson T (1991) Production and analysis of intraspecific somatic hybrids of potato (*Solanum tuberosum* L.). Plant Sci. 75: 107-115.

Weber DC and Ferro DN (1993) Distribution and overwintering Colorado potato beetle in and near Massachusetts potato fields. Entomol. Exp. Appl. 66: 191-196.

Weber DC, Ferro DN, and Stoffolano Jr. JC (1993) Quantifying flight of Colorado potato beetle (Coleoptera: Chrysomelidae) with a microcomputer-based flight mill system. Ann. Entomol. Soc. Am. 86: 366-371.

Weeden NF and Robinson RW (1986) Allozyme segregation ratios in the interspecific cross *Cucurbita maxima* X *C. ecuadorensis* suggest that hybrid breakdown is not caused by minor alterations in chromosome structure. Genetics 114: 593-609.

Wenzel O, Scheider O, Przewozny T, Sopory SK, and Melchers G (1979) Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor. Appl. Genet. 55: 49-55.

Wenzel G and Uhrig H (1981) Breeding for nematode and virus resistance in potato via anther culture. Theor. Appl. Genet. 59: 333-340.

Werner JE and Peloquin SJ (1991) Potato haploid performance in 2X x 4X crosses. Amer. Potato J. 68: 801-811.

Wetherell DF and Dougall DK (1976) Sources of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue. Physiol. Plant. 37: 97-103.

Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, and Miller JR (1993) Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 226-233.

Wierenga JM and Hollingworth RM (1993) Inhibition of altered acetycholinesterase from insecticide-resistant Colorado potato beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86: 673-679.

Yerk GL and Peloquin SJ (1990) Performance of haploid X wild species, 2x hybrids (involving five newly evaluated species) in 4X X 2X families. Amer. Potato J. 67: 405-417.

Zehnder GW, Ghidiu GM, and Speese III J (1992) Use of the occurrence of peak Colorado potato beetle (Coleoptera: Chrysomelidae) egg hatch for timing of *Bacillus thuringiensis* spray applications in potatoes. J. Econ. Entomol. 85: 281-288.

Ziskind LA and Mityakina ON (1991) *Endovum puttleri* (Hymenoptera, Eulophidae), an introduced entomophage of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). Entomol. Rev. 70: 142-148.

CHAPTER TWO: ANTHER CULTURE OF *SOLANUM PHUREJA - S. CHACOENSE* HYBRIDS AND DIPLOIDIZATION OF MONOPLOID *SOLANUM PHUREJA - S. CHACOENSE* GENOTYPES

Introduction

Colorado potato beetle (Coleoptera, Chrysomelidae: *Leptinotarsa decemlineata* Say, CPB) is a serious defoliator in potato (*Solanum tuberosum* L.) production regions of the United States. Incorporation of resistance into the potato genome would benefit potato growers who would then be able to reduce expensive and environmentally dangerous pesticides.

The seriousness of CPB is increased due to strains that are resistant to several currently available organic and inorganic pesticides (Follett et al. 1993; Forgash 1985). This is illustrated by current USDA spraying recommendations, which may reach 12 per year in severe outbreaks, costing farmers between $75 and $100 million annually (Perlak et al. 1993). CPB damage is magnified by the fact that each adult female can lay up to 450 eggs, and there can be up to three generations per year (Ferro and Boitreau 1993). Furthermore, many currently effective chemicals are being withdrawn from the market due to environmental hazards posed by their excessive buildup in the environment.

Further complications arise in breeding efforts because it has been shown that CPB can derive resistance to *Bacillus thuringiensis* (BT) (Whalon et al. 1993), the

basis of a recently touted control protocol (Ghidiu and Zehnder 1993; Hough-Goldstein et al. 1991; Zehnder et al. 1992). Generation of CPB resistant *Solanum* plant materials would reduce the need for inorganic or organic chemical pesticide or BT application, which would reduce both environmental damage and production costs.

Biocontrol has primarily focused on various natural chemical (Arpaia and Ricchiuto 1993; Liu et al. 1991; Mendel et al. 1991; Rahimi and Carter 1993), cultural (Milner et al. 1992; Stoner 1993), and insect (Hough-Goldstein and Whalen 1993; Mityakina et al. 1993; Riechert and Bishop 1990; Ziskind and Mityakina 1991) agents. Resistance to CPB by breeding *S. tuberosum* with other *Solanum* has also been suggested (Bani-Aameur et al. 1991, 1993; Plaisted et al. 1992).

The development of interspecific hybrid CPB resistant plant material may be an achievable goal. Two particular species of interest in this effort are *Solanum chacoense* (*chc*) and *S. berthaultii*. *S. berthaultii* produces what are called type B trichomes (Plaisted et al. 1992). These trichomes secrete sticky exudates which trap insects. *S. chacoense* synthesizes several unusual glycoalkaloids, including acetylleptinidine, that confer resistance to CPB (Deahl et al. 1993). Inheritance for alkaloid expression in *chc* is complex, as shown by McCollum and Sinden (1979). Leptine glycoalkaloid production is variable within the species, and even within single accessions of the species, such that high alkaloid synthesizing clones are rare (Sinden et al. 1986a). Identification of these rare high synthesizing clones is worth the effort involved, however, in that these alkaloids have been shown to be efficient deterrents to

CPB feeding and effectively lethal to all CPB life stages that feed (Harrison 1987; Melville et al. 1985; Sinden et al. 1980).

The primary activity of the leptine glycoalkaloids seems to be controlled by the acetylation of carbon 23 of the chaconine and solanine steroid aglycone rings, a metabolic action which is achieved by leptine glycoalkaloid producers of *S. chacoense* (Sinden et al. 1986b). The product of this reaction, acetylleptinidine, it is suggested, affects the insects not only by sterilizing and causing death in adult males and females, but also by deterring feeding by interacting with chemosensory cells (Mitchell 1987). The advantage of this resistance is that the alkaloids are present in levels high enough in the foliage to ensure control, while remaining at safe levels for human consumption in the tuber tissue (Sanford et al. 1992). Furthermore, strains of CPB resistant to leptines may be less likely to develop than strains resistant to other pesticides or CRYIIIA toxin for a long period. Acquired resistance may require more of an alteration of metabolism and/or physiological structure to adapt to a general toxin (acetylleptinidine) than a very specific toxin (i. e., CRYIIIA toxin or pesticides).

Introgression of this system of resistance into *Solanum tuberosum* (2n=4x=48) can be facilitated by hybridizing *chc* (2n=2x=24) first with *S. phureja* (2n=2x=24) (*phu*), a cultivated species also native to South America (Bani-Aameur et al. 1991, 1993). Phu is better adapted than *chc* in terms of growth habit, seasonality, critical photoperiod for tuberization, tuber production, tuber dormancy, and other characters (Correll 1962; Owen et al. 1988a). *Chc* also possesses a relatively high degree of heat tolerance, a trait useful in expanding the effective production range of *S. tuberosum* if

it could likewise be introgressed (Reynolds and Ewing 1989). Following hybridization at the 2x level, the allelic diversity of the *S. tuberosum* genome would be expanded by introgressing *phu* and *chc* genes, which could increase yield on the basis of heterozygosity and/or heterosis (Bonierbale et al. 1993; Chase 1963; Clulow et al. 1991).

A potential difficulty is that some of the interspecific hybrids of *chc* and *phu* (PC or CP hybrids) appear to be unstable in growth habit, flowering, and growth period (Bani-Aameur et al. 1993). Their best performance has been observed during summer months in the greenhouse, when most *Solanum* species, including *phu* and *S. tuberosum* cultivars, do not grow satisfactorily due to heat stress. Performance at other times of the year and under other conditions has been unpredictable and generally unsatisfactory. This undesirable and unstable performance may be investigated by observing and taking advantage of sexual segregation present in pollen by extraction of androgenic monoploids from actively growing and flowering hybrids. Ideally, monoploids will be identified that are superior in traits such as vigor, as lethal genes will be expressed and cause death early in development (Uijtewaal 1987). This is a mechanism that has been referred to as the 'monoploid sieve' (Wenzel et al. 1979).

The experiments described herein address the derivation of monoploid genotypes from existing diploid hybrids by anther culture and the doubling of the chromosomal complement of these monoploid genotypes by leaf disc regeneration. An important reason for deriving a good system of anther culture and leaf disc regeneration is to determine whether it is feasible to derive monoploids from unstable

diploid hybrids via anther culture and to ascertain whether it is possible to select a range of genotypes with high acetylleptinidine production, similar to *chc*, and a more normal growth habit, similar to *phu*. that can be converted to doubled monoploids for hybridization with other diploid genotypes.

Materials and Methods

Experiment 1. Anther culture has been widely used in the genus *Solanum* to reduce chromosome numbers and derive breeding material (Birhman et al. 1994; Cappadocia and Ahmim 1988; Cappadocia et. al. 1984, 1986; Meyer et al. 1993; Owen et al. 1988a; Powell and Uhrig 1987; Rivard et al. 1989; Tiainen 1992a, 1992b, 1993; Uhrig 1985; Wenzel and Uhrig 1981; Wenzel et al. 1979). Several factors have been found to influence embryo production by cultivated potato anthers, including parent plant age, growing conditions, parent plant health, anther pretreatments such as heat or cold, sugar sources, culture conditions (Tiainen 1992a), growth regulators (Tiainen 1993), and so on. This experiment will attempt to determine whether the effect of various reduced nitrogen sources in the anther culture and regeneration media is stimulatory as seen in somatic embryogenesis of carrot (*Daucus carota*) (Kamada and Harada 1984a, 1984b; Wetherell and Dougall 1976). Flow cytometry will be utilized to analyze resulting plantlets for ploidy level (Bergounioux et al. 1986; Bharathan et al. 1994; Owen et al. 1988b; Ozais-Akins and Jarret 1994; Sharma et al. 1983; ten Cate and Ramulu 1987; Waara et al. 1991). This is a procedure significantly simpler than the

traditional root tip squash method (Sharma et al. 1983) or the later method of counting guard cell chloroplast numbers (Singsit and Veilleux 1991).

Plant materials and methods. Three interspecific hybrids, *chc* 80-1 x *phu* 1-3 #62 (CP1), *chc* 80-1 x *phu* 1-3 #66 (CP2), and *phu* AD 3-4 x *chc* 55-1 #62 (RPC3), were utilized in this study. These clones were selected based on their vigorous growth, prolific flowering, tuberization, and alkaloid production. Two or three plants of each genotype were grown in 3.8 l black plastic pots containing 1 sand : 1 Weblite (Weblite Co., P. O. Box 12887, Roanoke VA) : 1 Sunshine mix (Fison Horticulture Inc., Vancouver BC, Canada) during the summer of 1993. Weekly fertilization was applied containing 20:19:18 ($N:P_2O_5:K_2O$) (Peter's Fertilizer Products, W. R. Grace & Co., Fogelsville, PA) at a rate of 400 mg/l. Flower buds were selected such that the majority of microspores were at the late uninucleate stage (anthers approx. 3 mm in length). Buds were placed in paper towels moistened with distilled water and stored in darkness at 4° C for 72 h. Either 30 or 60 buds were collected per day for a total of 90 buds per clone. Buds, in groups of 30, were surface sterilized in 70% ethanol for 30 sec, followed by disinfection for five min in 100% commercial bleach (Wonder bleach, 5.25% sodium hypochlorite). Buds were then rinsed three times in sterile distilled water. An anther from each bud was then placed in a 125 ml Erlenmeyer (Corning Glass Works, Corning, NY 14831) culture flask containing 15 ml of one of five reduced nitrogen variants of anther culture medium **(medium 1 Appendix A)** (Uhrig 1985) **(Table 2-1)** according to the 'different' scheme of Snider and Veilleux

(1994). Each treatment was adjusted to contain 30 μM nitrogen. Each flask was covered with a 2-way magenta cap (Magenta Plastics, Chicago, IL) and sealed with parafilm. Flasks were randomly placed on a rotary shaker, set to 120 rpm, in complete darkness at a temperature of approx. 20-21°C. The experimental design for the entire experiment was a randomized complete design. For each genotype, a total of 450 anthers was cultured. After six weeks, embryos were harvested. Embryo production per flask were analyzed by PROC GLM on the square root of embryos produced (SAS 1985). This analysis was utilized to normalize the extreme variation in embryo production between flasks and better observe treatment affects.

Anther-derived embryos were placed on 15 ml of B_5 regeneration medium (Gamborg et al. 1968) supplemented with containing 50 mg/l $CaHPO_4$ and 0.1 mg/l filter-sterilized GA_3 (**medium 2 Appendix A**) in 100 x 15 mm petri plates (Falcon® 1029, Becton Dickson and Co., Lincoln Park, NJ 07035), at a density of up to 30/plate. For each embryo, the nitrogen source used in the anther culture medium was retained in the regeneration medium at 30 μM. Plates were incubated in a high light (450 $\mu E/m^2/s$) incubator with a 16 h photoperiod and constant 20°C temperature. All light measurements were conducted with a light meter (LI-COR Quantum Radiometer Photometer, model LI-185 B, LI-COR, Inc.). Shoots from regenerated embryos were placed in 25 x 150 mm culture tubes (Pyrex® rimless culture tubes, Corning Glass Works, Corning, NY, 14831) with ≈17 ml of Murashige and Skoog (MS) basal medium **(medium 3 Appendix A)** (Murashige and Skoog 1962) and placed in a lower

light incubator (48 $\mu E/m^2/s$), also with a 16 h photoperiod and a constant ≈20° C temperature. After growth had occurred to allow removal of sufficient leaf and/or stem tissue for testing, 0.5 g of tissue was minced in a glass petri dish containing 1.5 ml chopping buffer (3.5 g/l sodium citrate, 1.7 g/l MOPS, 3.7 g/l $MgCl_2$, and 0.4 g/l Triton X-100) for three min. over ice. The resulting material was then filtered successively through 250 μM and 63 μM filters. Samples were stored in centrifuge tubes until all samples were prepared. RNA was digested by adding 0.25 ml RNAase solution (40 mg/50 ml chopping buffer) to 0.5 ml filtrate (Ribonuclease A, type 1-AS bovine pancreas, Sigma Chemical Co., St. Louis, MO, 63178). After 30 min. incubation at room temperature, DNA was stained by adding 0.125 ml propidium iodide (40 mg/100 ml chopping buffer) to the filtrate-RNAase solution. Samples were then returned to ice, to be analyzed by flow cytometry within a period of 30 min. to three h. For flow cytometric analysis, samples were further filtered through a 37 μM nylon mesh filter and analyzed using an Epics V, Model 752 laser flow cytometer and cell sorter (Coulter Electronics, Hialeah, FL). Relative DNA fluorescence of 10,000 nuclei was performed with laser excitation of 300 mW at 488 nm (5 W Innova 90 Argon Laser, Coherent Inc., Palo Alto CA). Histogram analysis of the data was according to Owen et al. (1988b).

Experiment 2. This experiment addressed the process of doubling the chromosome number of androgenic monoploids derived from interspecific PC hybrids. The purpose of this experiment was to derive homozygous diploid breeding material. The

procedures were analogous to those used successfully with *S. tuberosum* genotypes (Hulme et al. 1992) and were similar to procedures used by Fleming (1992). In addition, this experiment evaluated the effects of silver thiosulfate (STS) at various stages of the chromosome doubling process. STS, an ethylene synthesis inhibitor, has been shown to increase leaf expansion of *in vitro* potato plantlets (Perl et al. 1988) and to stimulate recovery of plantlets with a doubled chromosome content (Mollers et al. 1992).

Plant materials and methods. Intact leaflets of 11 monoploids derived by anther culture of PC hybrids (CP2-3, CP2-4, CP2-7, RPC 1-1, RPC 2-1, RPC 3-1, RPC 3-2, RPC 3-6, RPC 3-7, RPC 3-8, and RPC 3-9) and a *chc* anther-derived monoploid (C 8-4) were utilized in this experiment. Four plantlets of each genotype were propagated *in vitro* for three weeks, two plantlets on ≈17 ml of MS basal medium (Pyrex® rimless culture tubes, 25 x 150 mm, Corning Glass Works, Corning, NY, 14831) and two plantlets on ≈17 ml of MS basal medium supplemented with 1.5 mg/l silver thiosulfate (STS). Incubation was at 16 h photoperiod at ≈20° C, with a light intensity of 48 $\mu E/m^2/s$. Four treatments were imposed on each genotype, utilizing two pair of leaflets per plantlet, resulting in two replications for each genotype in each treatment, as illustrated in **Figure 2-1**. Leaflets were excised and placed in a high growth regulator pulse medium with 9.9 mg/l (44 μM) BA and 10.05 mg/l (54 μM) NAA (**medium 4 Appendix A**) for 48 h in the incubation conditions

stated above. At this point, leaflets were placed on 100 x 15 mm quad plates (Falcon® 1009, Becton Dickson and Co., Lincoln Park, NJ 07035) containing 2.25 mg/l (10 μM) BA and 0.18 mg/l (0.1 μM) IAA to induce callus formation (**medium 5 Appendix A**). These plates were used to minimize the spread of contamination. Two leaflets were placed per well, each of which contained 5 ml of media. After one week, plantlets were transferred to the regeneration medium, containing 2.25 mg/l (10μM) BA and 4.85 (14μM) mg/l GA_3 (**medium 6 Appendix A**), again in quad plates as described above, either containing or not containing 1.5 ml/l STS. Thus, the four treatments were: 1) absence of STS during the entire regeneration process, 2) STS present in both the propagation and regeneration medium, 3) STS present in the propagation medium but not the regeneration medium, and 4) STS present in the regeneration medium but not the propagation medium. Leaflets were transferred bi-weekly for a total culture period on the regeneration medium of 12 weeks. Shoot production was determined at each biweekly transfer. A maximum of ten shoots was retained from each genotype, regardless of treatment. These shoots were propagated and tested for ploidy as described for experiment 1. The effects of treatment, genotype, and interactions were determined using PROC GLM (SAS 1985). Mean total shoot production per disc and diploid frequency per leaf disc were analyzed using SNK.

Results

Experiment 1. Clone, treatment, and the clone*treatment interaction significantly affected embryo production in anther cultures of PC hybrids (**Table 2-2**). Clone CP2 produced approximately three times as many embryos as did clone CP1 and 30 times as many as RPC3 (**Table 2-3**). Regarding reduced nitrogen source, the control treatment of inorganic N (NH_4NO_3 and KNO_3) yielded the best results (**Table 2-4**). Neither of the NH_4Cl treatments yielded embryos. Casein hydrolysate, a complex and undefined source of amino acids, yielded significantly fewer embryos than the control. Only nine embryos converted into plants; all nine were diploid. Changing pH over the duration of culture was noticed in all treatments, with the least drift occurring in the glutamine treatment (5.6 to 5.9) and the most occurring in the casein hydrolysate treatment (5.6 to 4.3). A lowering of pH was also observed in the control (5.6 to 4.6) and the NH_4Cl + disodium succinate treatment (5.6 to 4.8), whereas the NH_4Cl + glutamic acid treatment exhibited a pH increase (5.6 to 6.0). The genotype by treatment interaction indicates that the genotypes behaved differently, specifically with CP2 outperforming CP1 by approximately 3 times and RPC3 by approximately 20 times.

Experiment 2. **Table 2-5** shows that treatment and the genotype*treatment interaction significantly affect total shoot production per leaf disc, while nothing else in the model did. Of the 11 genotypes tested, five produced no shoots at all. The remaining six varied in shoot production from 0.25 to 0.63 per leaf disc; however these differences

were not significant (**Table 2-6**). **Table 2-7** shows that STS in both the propagation and regeneration medium reduced shoot production and diploid frequencies. STS in the regeneration medium reduced the frequency of diploid shoots over the control.

Although the total shoot production per leaf disc is an important indicator of regeneration potential, the frequency of diploid shoots among total shoots regenerated is important because the purpose of the procedure is to derive homozygous diploid regenerants from monoploid sources. Disregarding genotype and treatments, 17 monoploid, 25 diploid, and 2 tetraploid shoots were regenerated. **Table 2-5** shows that genotype, treatment, and the genotype*treatment interaction were significant in altering the frequency of diploid shoots produced by leaf discs, with treatment being very significant. Genotype RPC 1-1 produced the highest frequency of diploid shoots per leaf disc (0.41), significantly more than genotype C 8-4 which produced diploids at the lowest frequency (0.05) (**Table 2-6**).

Discussion

Somatic embryogenesis is the capacity of plants to generate embryos from tissue other than a fertilized egg. These embryos can arise from vegetative tissue, reproductive tissue other than the fertilized egg, or portions of an embryo. Somatic embryogenesis can be used as a means of clonal propagation in several woody (Wann 1989) and herbaceous (Gray 1987) genera. Production of plants by somatic

embryogenesis in *S. tuberosum* (Pretova and Dedicova 1992; Reynolds 1986) has been performed, but this study obtained somatic embryos from zygotic embryos.

Androgenesis would fall into the second of the above described types of somatic embryogenesis, i. e. embryogenesis from reproductive tissue. Use of this type of somatic embryogenesis attempts to take advantage of genetic variation present in microspores to derive haploid material for breeding purposes. Factors affecting other types of somatic embryogenesis, such as reduced nitrogen source (Kamada and Harada 1984a, 1984b; Tiainen 1992b; Wetherell and Dougall 1976), could be expected to influence anther culture responses. In these studies in particular, casein hydrolysate, glutamine, and other nitrogen rich amino acids alone or a few in combination elicited superior results. In the experiment described here, alternative reduced nitrogen sources were inhibitory rather than stimulatory to androgenesis. None of the treatments appears profitable for increasing the anther culture response in this germplasm. Drift in pH, which has been observed to occur with the use of some reduced nitrogen treatments (Wetherell and Dougall 1976), was both higher and lower from the initial levels and did not seem to have a general effect on anther culture response. As is often true of tissue culture systems, there was a highly significant interaction between genotype and treatment. Thus, the genotypes were reacting differently to the same treatments and different treatments may have elicited the optimal results in the three genotypes.

In the leaf disc regeneration experiment, existing monoploids were utilized to evaluate the effect of silver thiosulfate on chromosome doubling. STS has been

reported to promote leaf expansion (Mollers et al. 1992; Perl et al. 1988) and play a role in endopolyploidization (Fleming et al. 1992; Hulme et al. 1992), the doubling of chromosomes during callus growth by chromosomal doubling without concomitant cellular division (Owen et al 1988b; ten Cate and Ramulu 1987). Taking advantage of endoploidization would reduce the need to use colchicine, which is toxic, does not penetrate tissue well (Fleming et al. 1992), and prolongs the tissue culture process with increased potential for somaclonal variation (Mitten et al. 1990; M'Ribu and Veilleux 1991). In the results presented here, not all genotypes responded uniformly to the doubling procedure, with several not forming shoots, and those forming shoots producing varying frequencies of diploids. All STS treatments produced a lower frequency of diploid shoots per leaf disc than the control. These results suggest that, at least for this germplasm, STS was in some way detrimental to organogenesis. This inhibition outweighed the reported beneficial effects of STS, namely ethylene suppression and leaf expansion for chromosomal doubling.

In conclusion, diploid interspecific hybrids can be used as sources of monoploid genotypes by means of anther culture. These monoploid genotypes can serve as the source of instantly derived inbred diploid lines by means of leaf disc regeneration of isolated diploid cells present naturally within monoploids due to endopolyploidization. For both total and diploid shoot production, genotype interacted with treatment. As diploid shoot recovery is of importance in this procedure, the fact that the interaction was so significant (P=0.0012) may be a problem for deriving an optimized system for all potato genotypes.

Literature Cited

Arpaia S and Ricchiuto B (1993) Effects of *Bacillus thuringiensis* toxin extracts on feeding behavior and development of Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 22: 334-338.

Bani-Aameur F, Lauer FI, and Veilleux RE (1993) Enhancement of diploid *Solanum chacoense* Bitt. using adapted clones of *Solanum phureja* Juz & Buk. Euphytica 68: 169-179.

Bani-Aameur F, Lauer FI, Veilleux RE, and Hilali A (1991) Genomic composition of 4x-2x potato hybrids: influence of *Solanum chacoense.* Genome 34: 413-420.

Bergounioux C, Perennes C, Miege C, and Gadal P (1986) The effect of male sterility on protoplast division in *Petunia hybrida.* Cell cycle comparison by flow cytometry. Protoplasma 130: 138-144.

Bharathan G, Lambert G, and Galbraith DW (1994) Nuclear DNA content of monocotyledons and related taxa. Amer. J. Bot. 81: 381-386.

Birhman RK, Rivard SR, and Cappadocia M (1994) Restriction fragment length polymorphism analysis of anther-culture-derived *Solanum chacoense.* HortScience 29: 206-208.

Bonierbale MW, Plastid RL, and Tanksley SD (1993) A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. Theor. Appl. Genet. 86: 481-491.

Cappadocia M and Ahmim M (1988) Comparison of two culture methods for the production of haploids by anther culture in *Solanum chacoense.* Can. J. Bot. 66: 1003-1005.

Cappadocia M, Cheng DSK, and Ludlum-Simonette R (1986) Self-incompatibility in doubled haploids and their F_1 hybrids, regenerated via anther culture in self-incompatible *Solanum chacoense* Bitt. Theor. Appl. Genet. 72: 66-69.

Cappadocia M, Cheng DSK, and Ludlum-Simonette R (1984) Plant regeneration from *in vitro* culture of anthers of *Solanum chacoense* Bitt. and interspecific diploid hybrids of *S. tuberosum* L. x *S. chacoense* Bitt. Theor. Appl. Genet. 69: 139-143.

Chase SS (1963) Analytic breeding in *Solanum tuberosum* L. -- a scheme utilizing parthenotes and other diploid stocks. Can. J. Genet. Cytol. 5: 359-363.

Clulow SA, Wilkinson MJ, Waugh R, Baird E, DeMaine MJ, and Powell W (1991) Cytological and molecular observations on *Solanum phureja*-induced dihaploid potatoes. Theor. Appl. Genet. 82: 545-551.

Correll DS (1962) The Potato and its Wild Relatives. Texas Research Foundation. Renner, Texas. 606 pp.

Deahl KL, Cantelo WW, Sinden SL, and Sanford LL (1991) The effect of light intensity on Colorado potato beetle resistance and foliar glycoalkaloid concentration of four *Solanum chacoense* clones. Amer. Potato J. 68: 659-666.

Deahl KL, Sinden SL, and Young RJ (1993) Evaluation of wild tuber-bearing *Solanum* accessions for foliar glycoalkaloid level and composition. Amer. Potato J. 70: 61-69.

Ferro DN and Boiteau G (1993) Management of insect pests. In: Rowe RC (Ed.) (1993) Potato Health Management. APS Press. St. Paul. pp. 103-108.

Fleming MLMH, De,Maine MJ, and Powell W (1992) Ploidy doubling by callus culture of potato dihaploid leaf explants and the variation in regenerated plants. Ann. Appl. Biol. 121: 183-188.

Follett PA, Gould F, and Kennedy GG (1993) Comparative fitness of three strains of Colorado potato beetle (Coleoptera: Chrysomelidae) in the field: spatial and temporal variation in insecticide selection. J. Econ. Entomol. 86: 1324-1333.

Forgash AJ (1985) Insecticide resistance in the Colorado potato beetle. In: Ferro DN and Voss RH (Eds.) Proceedings of the Symposium on the Colorado potato beetle, 17th Congress of Entomology, 1984. pp. 33-52.

Gamborg OL, Miller RA, and Ojimal L (1968) Nutrient requirements of suspension cultures of soybean root cells. Exptl. Cell Res. 50: 151-158.

Ghidiu GM and Zehnder GW (1993) Timing of initial spray application of *Bacillus thuringiensis* for control of the Colorado potato beetle (Coleoptera: Chrysomelidae) in potatoes. Biol. Cont. 3: 348-352.

Gray D (1987) Quiescence in monocotyledonous and dicotyledonous somatic embryos induced by dehydration. HortScience 22: 810.

Harrison GD (1987) Host-plant discrimination and evolution of feeding preference in the Colorado potato beetle *Leptinotarsa decemlineata*. Physiol. Entom. 12: 407-415.

Hough-Goldstein J and Whalen J (1993) Inundative release of predatory stink bugs for control of Colorado potato beetle. Biol. Cont. 3: 343-347.

Hough-Goldstein J, Tisler AM, Zehnder GW, and Uyeda KA (1991) Colorado potato beetle (Coleoptera: Chrysomelidae) consumption of foliage treated with *Bacillus thuringiensis* var. *san diego* and various feeding stimulants. J. Econ. Entomol. 84: 87-93.

Hulme JS, Higgins ES, and Shields R (1992) An efficient genotype-independent method for regeneration of potato plants from leaf tissue. Plant Cell Tiss. Organ Cult. 31: 161-167.

Kamada H and Harada H (1984a) Studies on nitrogen metabolism during somatic embryogenesis in carrot. I. Utilization of α-alanine as a nitrogen source. Plant Sci. Lett. 33: 7-13.

Kamada H and Harada H (1984b) Changes in endogenous amino acid compositions during somatic embryogenesis in *Daucus carota* L. Plant & Cell Physiol. 25: 27-38.

Liu Y, Alford AR, and Bentley MD (1991) Changes in antifeedant activity of limonin double salt in potato leaves. J. Econ. Entomol. 84: 1154-1157.

McCollum GD and Sinden SL (1979) Inheritance study of tuber glycoalkaloids in a wild potato, *Solanum chacoense* Bitter. Amer. Potato J. 56: 95-113.

Melville AA, Storch RH, Bushway RJ, and Alford AR (1985) Growth and development of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), fed foliage of three *Solanum* species. Maine Ag. Exp. St. Tech. Bull. 115.

Mendel MJ, Alford AR, Rajab MS, and Bentley MD (1991) Antifeedant effects of citrus limonoids differing in A-ring structures on Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 84: 1158-1162.

Meyer R, Salamini F, and Uhrig H (1993) Isolation and characterization of potato diploid clones generating a high frequency of monohaploid or homozygous diploid androgenetic plants. Theor. Appl. Genet. 85: 905-912.

Milner M, Kung KS, Wyman JA, Feldman J, and Nordheim E (1992) Enhancing overwintering mortality of Colorado potato beetle (Coleoptera: Chrysomelidae) by manipulating the temperature of its diapause habit. J. Econ. Entomol. 85: 1701-1708.

Mitchell BK (1987) Interactions of alkaloids with galeal chemosensory cells of Colorado potato beetle. J. Chem. Ecol. 13: 2009-2022.

Mitten DH, Horn M, Burrell MM, and Blundy KS (1990) Strategies for potato transformation and regeneration. In: Vayda ME and Park WD (Eds.) The Molecular and cellular biology of the potato. C. A. B. International. Oxford, UK. pp. 181-191.

Mityakina ON, Ziskind LA, Izhavski SS, and Klimenko A (1993) The effects of temperature and humidity on development of *Edovum puttleri* (Hymenoptera, Eulophidae), an egg parasite of Colorado potato beetle. Zoologichesky Zhurnal 72: 118-124.

Mollers C, Zhang S, and Wenzel G (1992) The influence of silver thiosulfate on potato protoplast cultures. Plant Breeding 108: 12-18.

M'Ribu HK and Veilleux RE (1991) Phenotypic variation and correlations between monoploids and doubled monoploids of *Solanum phureja*. Euphytica 54: 279-284.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Owen HR, Veilleux RE, Haynes FL, and Haynes KG (1988a) Photoperiod effects on 2n pollen production, response to anther culture, and net photosynthesis of a diplandrous clone of *Solanum phureja*. Amer. Potato J. 65: 131-139.

Owen HR, Veilleux RE, Levy D, and Ochs DL (1988b) Environmental, genotypic, and ploidy effects on endopolyploidization within a genotype of *Solanum phureja* and its derivatives. Genome 30: 506-510.

Ozias-Akins P and Jarrey RL (1994) Nuclear DNA content and ploidy levels in the genus *Ipomoea*. J. Amer. Soc. Hort. Sci. 119: 110-115.

Percival GC, Harrison JAC, and Dixon GR (1993) The influence of temperature on light enhanced glycoalkaloid synthesis in potato. Ann. Appl. Biol. 123: 141-153.

Perl A, Aviv D, and Galun E (1988) Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. Plant Cell Rep. 7: 403-406.

Perlak FJ, Stone TB, Muskopf YM, Petersen LJ, Parker GB, McPherson SA, Wyman J, Love S, Reed G, Biever D, and Fischoff DA (1993) Genetically improved potatoes: protection from damage by Colorado potato beetle. Plant Mol. Biol. 22: 313-321.

Plaisted RL, Tingey WM, and Steffens JC (1992) The germplasm release of NYL 235-4, a clone with resistance to the Colorado potato beetle. Amer. Potato J. 69: 843-846.

Powell W and Uhrig H (1987) Anther culture of *Solanum* genotypes. Plant Cell Tiss. Org. Cult. 11: 13-24.

Pretova A and Dedicova B (1992) Somatic embryogenesis in *Solanum tuberosum* L. cv. Desiree from unripe zygotic embryos. J. Plant Physiol. 139: 539-542.

Rahimi FR and Carter CD (1993) Inheritance of zingiberene in *Lycopersicon*. Theor. Appl. Genet. 87: 593-597.

Reynolds MP and Ewing EE (1989) Heat tolerance in tuber bearing *Solanum* species: a protocol for screening. Amer. Potato J. 66: 63-73.

Reynolds TL (1986) Somatic embryogenesis and organogenesis from callus cultures of *Solanum carolinense*. Amer. J. Bot. 73: 914-918.

Riechert SE and Bishop L (1990) Prey control by an assemblage of generalist predators: spiders in garden test systems. Ecology 71: 1441-1450.

Rivard SR, Cappadocia M, Vincent G, Brisson N, and Landry BS (1989) Restriction fragment length polymorphism (RFLP) analyses of plants produced by *in vitro* anther culture of *Solanum chacoense* Bitt. Theor. Appl. Genet. 78: 49-56.

Sanford LL, Deahl KL, Sinden SL, and Ladd Jr. TL (1992) Glycoalkaloid contents in tubers from *Solanum tuberosum* populations selected for potato leafhopper resistance. Amer. Potato J. 69: 693-703.

SAS Institute Inc. (1985) SAS user's guide: statistics. SAS Institute Inc., Cary, NC.

Sharma E, Firoozabady E, Ayers NW, and Galbraith DW (1983) Improvement of anther culture in *Nicotiana*: medium, culture conditions and flow cytometric determination of ploidy levels. Z. Plflanzenphysiol. 111: 441-451.

Sinden SL, Sanford LL, and Deahl KL. (1986a) Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter. J. Agric. Food Chem. 34: 372-377.

Sinden SL, Sanford LL, Cantelo WW, and Deahl KL. (1986b) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in *Solanum chacoense*. Environ. Entomol. 15: 1057-1062.

Sinden SL, Sanford LL, and Osman SF (1980) Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* Bitter. Amer. Potato J. 57: 331-343.

Singsit C and Veilleux RE (1991) Chloroplast density in guard cells of leaves of anther-derived potato plants growing *in vitro* and *in vivo*. HortScience 26: 592-594.

Stoner KA (1993) Effects of straw and leaf mulches and trickle irrigation on the abundance of Colorado potato beetles (Coleoptera: Chrysomelidae) on potato in Connecticut. J. Entomol. Sci. 28: 393-403.

Ten Cate CHH and Ramulu KS (1987) Callus growth, tumor development and polyploidization in the tetraploid potato cultivar Bintje. Plant Sci. 49: 209-216.

Tiainen T (1992a) The influence of culture conditions on anther culture response of commercial varieties of *Solanum tuberosum* L. Plant Cell Tiss. Org. Cult. 30: 211-219.

Tiainen T (1992b) The role of ethylene and reducing agents on anther culture response of tetraploid potato (*Solanum tuberosum* L.). Plant Cell Rep. 10: 604-607.

Tiainen T (1993) The influence of hormones on anther culture response of tetraploid potato (*Solanum tuberosum* L.) Plant Sci. 88: 83-90.

Uhrig H (1985) Genetic selection and liquid medium conditions improve the yield of androgenic plants from diploid potatoes. Theor. Appl. Genet. 71: 455-460.

Uijtewaal BA (1987) The procedure and evaluation of monoploid potatoes (2n=x=12) for breeding research on cell and plant level. Euphytica 36: 745-753.

Waara S, Wallin A, and Eriksson T (1991) Production and analysis of intraspecific somatic hybrids of potato (*Solanum tuberosum* L.). Plant Sci. 75: 107-115.

Wann SR (1989) Somatic embryogenesis in woody species. Hort. Rev. 10: 153-181.

Wenzel G and Uhrig H (1981) Breeding for nematode and virus resistance in potato via anther culture. Theor. Appl. Genet. 59: 333-340.

Wenzel G, Scheider O, Przewozny T, Sopory SK, and Melchers G (1979) Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor. Appl. Genet. 55: 49-55.

Wetherell DF and Dougall DK (1976) Sources of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue. Physiol. Plant. 37: 97-103.

Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, and Miller JR (1993) Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 226-233.

Zehnder GW, Ghidiu GM, and Speese III J (1992) Use of the occurrence of peak Colorado potato beetle (Coleoptera: Chrysomelidae) egg hatch for timing of *Bacillus thuringiensis* spray applications in potatoes. J. Econ. Entomol. 85: 281-288.

Ziskind LA and Mityakina ON (1991) *Endovum puttleri* (Hymenoptera, Eulophidae), an introduced entomophage of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). Entomol. Rev. 70: 142-148.

Table 2-1. Reduced nitrogen sources supplemental to liquid anther culture medium.

treatment	reduced nitrogen source
1	NH_4NO_3 + KNO_3 (control)
2	casein hydrolysate (13% N)
3	glutamine
4	NH_4Cl + disodium succinate (2:1 ratio NH_4Cl:disodium succinate)
5	NH_4Cl + glutamic acid (1:1 ratio NH_4Cl:glutamic acid)

Table 2-2. ANOVA for androgenic embryo production by three diploid potato clones in five reduced nitrogen treatments, based on the square root of embryos produced.

source	df	mean squares	F value	Pr > F
clone	2	47.16	52.32	0.0001
treatment	4	114.86	63.72	0.0001
clone*treatment	8	60.31	16.73	0.0001
replication (clone)	7	5.20	1.65	0.1738

Table 2-3. Mean embryos produced per flask for three clones of PC hybrids. Analysis was performed on the square root of embryos produced.

clone	n	mean
CP2	13	17.5 a
CP1	17	5.0 b
RPC3	14	0.6 c

*note: means followed by the same letter are not statistically different using SNK ($P<0.05$).

Table 2-4. Mean androgenetic embryo production per flask of 30 anthers by three PC clones, as affected by reduced nitrogen source.

nitrogen treatment	n	mean
NH_4NO_3 + KNO_3	9	23.8 a
glutamine	7	9.1 b
casein hydrolysate (13% N)	8	5.4 b
NH_4Cl + disodium succinate (2:1 ratio NH_4Cl:disodium succinate)	10	0.0 c
NH_4Cl + glutamic acid (1:1 ratio NH_4Cl:glutamic acid)	10	0.0 c

*note: means followed by the same letter are not statistically different using SNK ($P<0.05$).

Table 2-5. ANOVA for total shoot and diploid shoot frequency from leaf discs of six monoploid potato clones on four regeneration schemes (see **Fig. 1.**).

source	df	mean squares	F value	Pr > F
total shoot production				
genotype	5	0.29	1.01	0.4217
treatment	3	2.47	8.31	0.0001
replication	1	1.02	3.77	0.0564
genotype*treatment	15	1.66	5.68	0.0001
diploid frequency				
genotype	5	0.22	2.32	0.0529
treatment	3	0.72	7.58	0.0002
replication	1	0.25	2.63	0.1095
genotype*treatment	15	0.28	2.97	0.0012

Table 2-6. Mean total shoot (1x, 2x, 4x) production and frequency of diploid shoots per cultured leaf disc among five PC monoploid genotypes and one *chc* monoploid.

monoploid genotype	n	mean	diploid frequency
CP 2-3	16	0.63 a	0.24 ab
RPC 1-1	16	0.56 a	0.41 a
C 8-4	16	0.50 a	0.05 b
RPC 3-9	16	0.50 a	0.16 ab
RPC 3-8	16	0.38 a	0.19 ab
RPC 3-6	16	0.25 a	0.25 ab

* note: means followed by the same letter are not statistically different using SNK ($P<0.05$).

Table 2-7. Mean shoot production and frequency of diploid shoots per cultured leaf disc by six monoploid genotypes on four regeneration schemes.

treatment	n	mean shoot production	diploid frequency
0 mg/l STS in prop. medium 0 mg/l STS in regen. medium	24	0.71 a	0.42 a
1.5 mg/l STS in prop. medium 0 mg/l STS in regen. medium	24	0.58 a	0.26 ab
0 mg/l STS in prop. medium 1.5 mg/l STS in regen. medium	24	0.58 a	0.18 b
1.5 mg/l STS in prop medium 1.5 mg/l STS in regen medium	24	0.00 b	0.00 c

* note: means followed by the same letter are not statistically different using SNK ($P<0.05$).

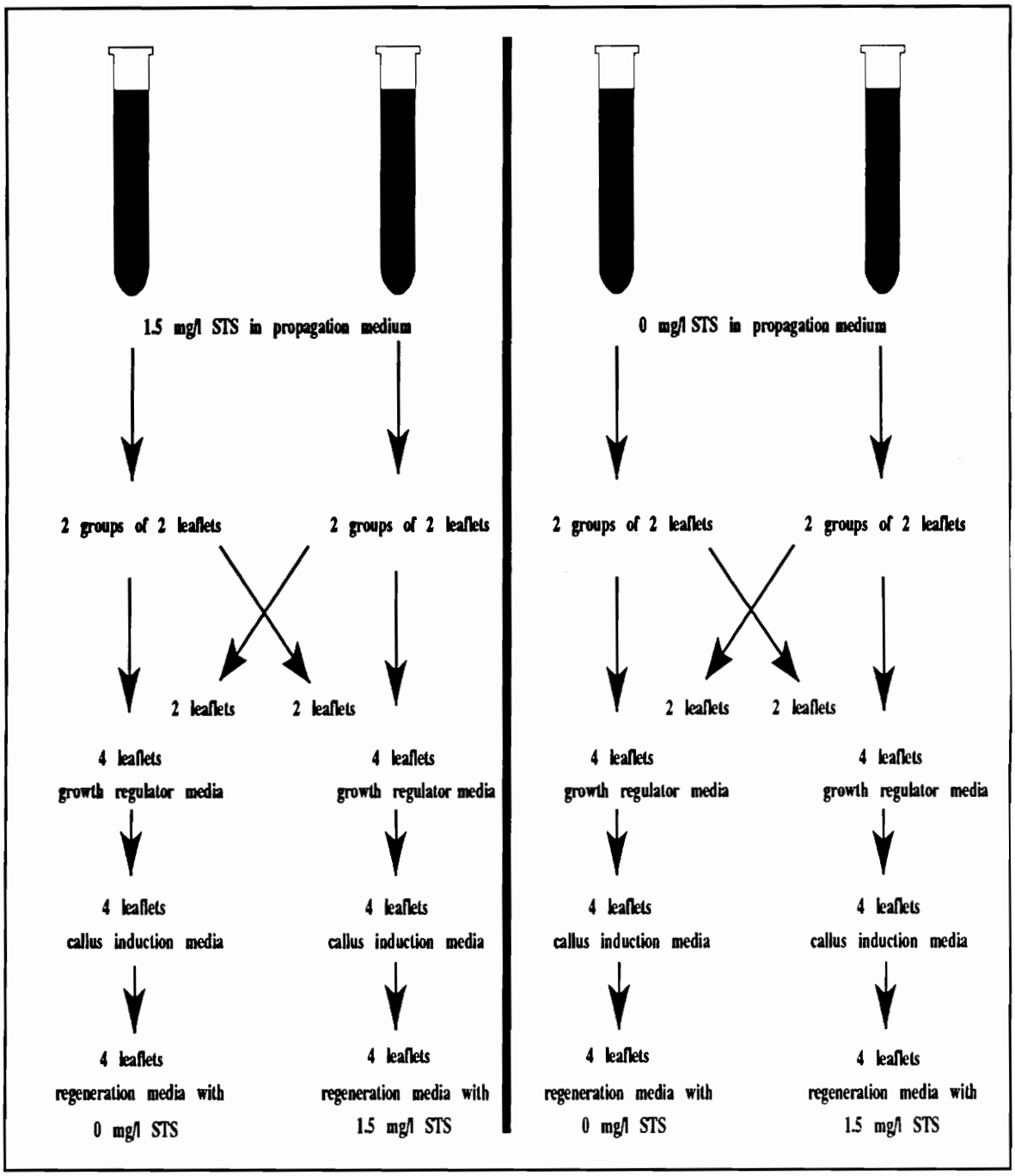

Figure 2-1. Distribution of 16 leaf discs per genotype onto four regeneration schemes in experiment 2.

CHAPTER THREE: HYBRID BREAKDOWN IN *SOLANUM CHACOENSE* X *S. PHUREJA* HYBRIDS

Introduction

Of the approximately 1000-2700 *Solanum* species (Correll 1962; D'Arcy 1979, 1991; Hawkes 1963; Rowe 1993), 160-180 tuberize and fewer than ten are utilized as major food products (Rowe 1993). Of these, *S. muricatum*, *S. melogena*, *S. quitoense*, *S. sessiliflorum*, and *S. tuberosum* are the most important (Bernardello et al. 1994; Spooner et al. 1993). The four former species are important for fruit production and the last is important for tuber production. *S. tuberosum* is grown on 44 million plus acres and yields in excess of 250 million tons in more than 125 countries, making it the fourth most important food crop in the world, after wheat, maize, and rice.

Yield is maximized for potato by control of fungal and viral diseases, nematodes, and insects. The Colorado potato beetle (Coleoptera, Chrysomelidae: *Leptinotarsa decemlineata* Say, CPB) is one of the most serious insect pests. This pest is endemic to most potato production regions of the world. The primary reasons for concern about CPB are its rapid reproduction, resistance to organic and inorganic pesticides, and its profound capability to generate resistance to biological controls. USDA spray recommendations may reach 12 per season in severe infestations, costing US growers anywhere between $75 and $100 million dollars (Perlak et al. 1993). Many pesticides registered for use on potatoes for CPB control are being withdrawn from the market, due to increasing CPB resistance (Bishop and Grafius 1991; Forgash 1985; Wierenga and Hollingworth 1993). In addition, chemicals still in use are

applied at higher levels with decreasing efficiency. The reproductive capabilities of CPB are impressive. A single CPB adult female may lay up to 450 eggs in her lifetime and there may be up to three complete CPB life cycles per year under favorable conditions (Ferro and Boiteau 1993). These conditions can lead to explosive buildups in beetle populations.

Solanum chacoense Bitt. (*chc*) (2n=2x=24) is a widely variable species native to the lowlands of South America, particularly Bolivia, Paraguay, Uruguay, Northern Argentina, and Southern Brazil (Correll 1962). It is a self-incompatible species (Cappadocia et al. 1986; Xu et al. 1990) that shows extreme inbreeding depression on selfing. *Chc* has been suggested as a breeding component of biocontrol against CPB (Bani-Aameur 1991, 1993; Carter 1987). Many alkaloids are synthesized by *chc* including some unusual ones (Osman et al. 1976). Among the most interesting are a specific type, characterized as leptine alkaloids. These are notable in having acetyl groups attached to carbon 23 of the chaconine and solanine steroid aglycone ring (Sinden et al. 1986b).

The primary alkaloid of interest produced by this reaction is acetylleptinidine (Deahl et al. 1993; Osman et al. 1987). This and related chemicals have been shown to be toxic to all stages of CPB feeding (Melville et al. 1985). Primarily, glycoalkaloid-based resistance has focused on introgression of alkaloid synthesis genes, but foliar sprays of leptine alkaloids have been suggested (Costa and Gaugler 1989). In either case, the alkaloids lead to death of beetle larvae and adults feeding on foliar tissue and also act as feeding deterrents to both larvae and adults (Sinden et al. 1980;

Harrison 1987). The mechanism of the latter of these functions is unclear, but it has been hypothesized to result from the interaction of volatilizing alkaloids with CPB galeal chemosensory cells (Mitchell 1987).

This particular glycoalkaloid synthesis pathway is especially attractive in breeding for resistance because these alkaloids are synthesized in high levels in foliar tissue while remaining low in tuber tissue (Sanford et al. 1992). This specific group of alkaloids has been found only in *chc* (Cantelo et al. 1987). Inheritance for production of leptine glycoalkaloids in *chc* is complex (McCollum and Sinden 1979). This is largely due to the fact that synthesis within an accession is variable, with most members, even in high producing accessions, synthesizing little or no leptine glycoalkaloids and relatively few individuals synthesizing high levels (Sinden et al. 1986a). Alkaloid production in these plants is further complicated by the fact that alkaloid synthesis in *chc* is influenced by temperature (Percival et al. 1993) and light (Deahl et al. 1991; Percival et al. 1993). Analysis of alkaloid production has been simplified by recently derived thin layer chromatography (TLC) techniques which allow screening of up to 80 foliage samples or more per day (Deahl and Sinden 1987).

S. chacoense has also been shown to be effective in control of potato leafhopper by means of these same alkaloids (Sanford and Ladd 1992; Sanford et al. 1992, 1994). In addition, *chc* is of interest due to its high heat tolerance (Reynolds and Ewing 1989), a trait useful for expansion of the *S. tuberosum* production range (Ewing 1981; Nowak and Colborne 1989). This species also has been characterized for its tissue culture competence, particularly in terms of anther culture competence

(Birhman et al. 1994; Cappadocia and Ahmim 1988; Cappadocia et al. 1986; Rivard et al. 1989; Xu et al. 1990). However, its usefulness in potato breeding programs may be somewhat limited due to its extremely weedy nature, characterized by massive stolon proliferation and variable production of small tubers (Correll 1962).

The use of *chc* in potato breeding has been suggested by many authors (Bani-Aameur et al. 1991; Cappadocia et al. 1984; Douches and Quiros 1988; Grun and Chu 1978; Jacobsoen and Jansky 1989; Mooney and Jansky 1990; Sanford and Ladd 1992; Summers and Grun 1981; Waara et al. 1991). Problems with hybrid stability involving *chc* have been cited in some of these studies (Bani-Aameur et al. 1991; Douches and Quiros 1988; Mooney and Jansky 1990; Summers and Grun 1981). Two of these studies cite usage of hybrids between *Solanum chacoense* and *S. phureja* (Bani-Aameur et al. 1991; Grun and Chu 1978).

S. phureja (*phu*) Juz. and Buk. is a more adapted species than *chc*. It is also native to South America where it is cultivated in Venezuela, Columbia, Ecuador, and northern Peru (Correll 1962). Although this species is quite variable, an adapted population with regard to photoperiod response, tuber size, tuber shape, and tuber production has been developed for breeding purposes (Haynes et al. 1972). Use of *phu* x *chc* (PC) hybrids for introgression of traits into *S. tuberosum* has been suggested as a means of improving yield on the basis of introduction of useful characters while maintaining allelic diversity (Bani-Aameur et al. 1991, 1993). Increased yield in *S. tuberosum* has been hypothesized to depend on maximizing heterozygosity (Bonierbale et al. 1993; Chase 1963).

In a previous study of PC hybrids, it was mentioned that 15 of 35 hybrid families were discarded due to a high frequency of weak plants. We have observed similarly weak PC hybrids in crosses between adapted *phu* clones and high leptine clones of *chc* (Bani-Aameur et al. 1991). This weakness is characterized by poor seed germination, slow growth of seedlings, or premature death of seedlings, especially during a cool season generally considered beneficial for potato growth. Vigorous growth of exceptional PC seedlings in otherwise weak families has been observed in hot greenhouse environments, generally considered undesirable for potato. At other times of the year, even with supplemental light and nutrients, these seedlings do not thrive. Such observations lead us to suspect that 'hybrid breakdown' or 'hybrid instability', similar to what has been observed in *Cucurbita* (Cutler and Whitaker 1969; Weeden and Robinson 1986), *Gossypium* (Harland 1936; Stephens 1949, 1950), *Platanus* (Sax 1933), *Campsis* (Sax 1933), and other genera (Grant 1966; Stebbins Jr. 1945) may be occurring in our PC hybrids. Generally, such instability has been attributed to mitotic and meiotic pairing irregularities, due to gross structural differences between the component parents of the hybrid (Sax 1933; Weeden and Robinson 1986). This disruption in cellular division usually results in a lack of viable embryos or seeds or death early in the life cycle of the hybrid.

The current study seeks to address the problem of hybrid breakdown of PC and CP hybrids observed in progenies of the hybrid clones by expanding the range of hybrids to include both leptine producing and non-leptine producing *chc* clones, all reported as CPB resistant, and more variable *phu* clones or related diploids, as parents.

Furthermore, field performance of such hybrids will be conducted to evaluate adaptation to local conditions and resistance to natural CPB infestations.

Materials and Methods

Crosses were performed in the greenhouse during three periods: November, 1992 to March, 1993; May, 1993 to July, 1993; and September, 1993 to December, 1993. Four types of crosses were attempted: *phu* (clones 13-14, 1-3, PP5, ID4, ID5, or ID8) X *chc* (clones 55-1, 80-1, PI's 133123, 175419, 209411, 275136, 414143, or 472817 H); *S. chacoense* (55-1, 80-1, or PI 472810 J) X *phu* (1-3, 13-14, or PP5); *chc* (55-1, 80-1, or PI 472810 I) X *chc* (PI's 133123, 175419, or 209411); and *phu* (ID4, ID5, or ID8) X *phu* (1-3, 13-14, or PP5). The ID (Idaho) clones are actually complex interspecific hybrids and have been utilized as a highly adapted 2x potato germplasm. ID4 (AD x 463-5) and ID5 (AD x 479-1) of composed of 1/2 *S. stenotomum*, 3/16 *phu*, 3/16 *S. tuberosum*, and 1/8 *chc*, although the clones used in generating each are different. ID8 (AD x 881-4) is composed of 7/16 *S. stenotomum* 11/32 *S. tuberosum*, and 7/32 *phu*. These clones were obtained from Dr. Joseph Pavek, USDA-ARS, Aberdeen, ID after several cycles of field selection. *chc* clones 55-1 and 80-1, obtained from Dr. Steven Sinden, USDA-ARS, Beltsville, MD, have been identified as high producers of the alkaloids solanidine, leptinidine, and acetylleptinidine. *chc* PI's 133123, 175419, 209411, 275136, 414143, and 472810 were obtained as seed from the Potato Introduction Station, NRSP-6, Sturgeon Bay, WI, 54235. All were reported by

Hanneman and Bamberg (1986) to be CPB resistant, but without mention of the mechanism. Ten plants grown from seed of each accession were bulk evaluated for solanidine, leptinidine, and acetylleptinidine by Dr. A. Raymond Miller at OARDC, Wooster, OH. Leaf samples for each accession were collected from each plant at mid stem, bulked, and freeze-dried for 72 h in a vacuum. An equal number of leaves from mid-canopy were selected from each tested plant and these leaves were bulked within accessions. Of these accessions, PI 472810 was the only accession to produce significant levels of all three alkaloids. Of the 10 individual plants within this accession, four produced no leptinidine or acetylleptinidine, three produced moderate levels of the alkaloids, and three produced high levels of the alkaloids.

All plants utilized for hybridization were grown in the greenhouse. Plants grown between October and March received 16 h supplemental light from high pressure sodium vapor lamps. Between two and four individual plants of clones used as pollen parents and between three and five individual plants of female clones were grown in 3.8 l black plastic pots. Pollen was collected from flowers by use of a flashlight modified with a stiff wire attachment that vibrated to shake pollen out of mature anthers. Pollen was collected on a clean sterile glass slide. Pollen was bulked among individuals of a clone or accession, for pollination as necessary. Either four or five flowers/inflorescence were emasculated for pollination. All plants were grown in a medium containing 1 sand: 1 Weblite (Weblite Co., P. O. Box 12887, Roanoke VA): 1 Sunshine mix (Fison Horticulture Inc., Vancouver BC, Canada). Weekly fertilization was applied containing 20:19:18 ($N:P_2O_5:K_20$) (Peter's Fertilizer Products,

W. R. Grace & Co., Fogelsville, PA) at a rate of 400 mg/l. Fruits were wrapped with cheesecloth at two weeks after pollination and were harvested after five or six weeks after pollination, when berries matured. Fruit was allowed to soften at room temperature for approximately one week. Seeds were extracted and dried for 24-48 h at room temperature. Pollinations per cross, fruits per cross, and seed per fruit for each cross are presented in **Table 3-1a** and **Table 3-1b**.

Seeds from each fruit were stored separately at approximately 5°C in white 5.7 cm x 9 cm coin envelopes (Craftmaster 1-A coin envelopes, #C-41112) until March 31, 1994. When available, 64 seeds per family were soaked in 10 ml distilled water containing 2000 ppm GA_3 (Sigma Chemical Co., St. Louis, MO, 63178) to break seed dormancy (D'Antonio and McHale 1988). Vessels with seedlings and GA_3 were placed on a Precision water bath shaker at 64-68 oscillations per minute (Precision Water Bath Shaker model 50, Precision Scientific Group, Chicago, IL 60647). On April 1, 1994, seedlings were placed under mist in 10 cm green plastic pots. Germinated seedlings were transplanted on a weekly basis for four weeks into 120 cell styrofoam cell packs containing the above described soil mix. Each cell measured 2.5 cm x 2.5 cm x 7.0 cm. Seedlings were observed and photographed weekly to record germination percentage, vigor, and development over time.

On April 29, 1994, field blocks at the Virginia Tech Whitethorne Kentland Agricultural research farm were fertilized. Soil in the blocks used in this study were characterized as a Hayter loam (fine loamy, mixed, mesic, Ultic Hapludalf). Blocks were broadcast fertilized with 10-20-20 ($N:P_2O_5:K_2O$), at a rate of 13.2 kg/30.48 m row.

S. tuberosum cvs. Superior and Kennebec were each planted as borders around two blocks as guard rows. *S. tuberosum* cvs. Desiree and Kahtadin were each planted within two blocks as breaks between the hybrid families. A total of 18 families was included in this study **(Table 3-3)**; seedlings were transplanted into the field blocks on May 18. *chc* clones 55-1 and 80-1 and *phu* clones 1-3 and 13-14 were planted as parental species checks, with 55-1 combined with 1-3 in one block and with 13-14 in a second block. The other two blocks contained either 80-1 and 1-3 or 80-1 and 13-14. From each hybrid family, 12 seedlings were used. Two blocks of three seedlings/cross each were not sprayed for CPB and two blocks of three seedlings/cross each were sprayed for CPB using Ambush at a rate of 147 g/ha **(Appendix B)**. All four blocks received fungicide treatments as described in **Appendix B**. Extra seedlings were maintained on raised greenhouse benches in 10 cm green plastic pots to generate tubers. These plants were maintained to keep representatives as free as possible from viruses that field grown plants were exposed to. Observations of both field and greenhouse plants focused on growth, vigor, days until flowering, natural fruit set mediated by bumble bees (in the field), total tuber weight, and tuber size. Field expression of resistance to natural CPB infestations was estimated with a visual rating of 0 (no damage) to 5 (severe to total defoliation) (Zehnder and Evanylo 1988), with two blocks sprayed as necessary for CPB control and two blocks not receiving any insecticide as control. A rank of 0 indicated no apparent damage, with a 5 representing total defoliation. A rank of 1 indicated 0-15% defoliation, with a 2 representing 15-50% defoliation, a 3 representing 50-85% defoliation and a 4

representing above 85-100% defoliation. Blocks were arranged in a split plot design with two treatments and two blocks per treatment. Plants were harvested September 12, 1994. Tubers were washed, counted and weighed on the same day. CPB damage at 4, 8, and 12 weeks after transplant and tuber data were analyzed using PROC GLM, with mean separations performed using LSD (SAS 1985). Models used a nested analysis with blocks nested within treatment and individual hybrid families nested within hybrid family type.

Results

Tables 3-1a and **3-1b** illustrate the difficulty in obtaining hybrids between, and to a degree within, these species. Of a total of 48 PC, 10 CP, 9 PP, and 10 CC hybrid combinations attempted, only 15, 4, 3, and 10 yielded fruit, respectively. The efficiency of fruit production for each of these family classes is presented in **Tables 3-1a** and **3-2**. Seed production per fruit was also extremely variable among the crosses that set fruit. **Table 3-3** presents the number of seed utilized in greenhouse and field evaluation of 30 hybrid families. As can be seen in **Table 3-4**, the germination percentage of the different types varied greatly, although there was no statistically significant difference between types using SNK. There was also considerable variation among the four types of families for seed number per fruit, but again, there were no statistically significant differences between the families (data not shown). In five families (80-1 x 209411, ID8 x 414143, ID8 x 133123, 80-1 x 175419, and 1-3 x

414143), no seeds germinated whereas in two others (13-14 x 275136 and 13-14 x 175419), there was 100% germination. **Table 3-4** presents combined plant survival at two and four weeks post transplant, flowering, and tuberization for field and greenhouse plants combined. This table also presents the number of plants alive at the selected points of time and compares to the theoretical maximum number of plants if all seed had germinated and presents the reduction in this value over time. **Tables 3-5** and **3-6** present plant survival at varying stages in the field and greenhouse, respectively. Full flowering was designated as occurring on August 5, 1994 within all blocks. This date was designated due to their being maximum flowering in most plots of all four blocks, compared to two weeks earlier and two weeks later. The decrease in plant number by this point from transplanting is presented in **Table 3-5**. Total plants among PP crosses decreased by 33%, among CP crosses by 25%, among CC crosses by 18%, and among PC crosses by 17.5%. **Tables 3-4** and **3-6** are incomplete as greenhouse plants didn't reach full flowering by the completion of this study. Three hybrid families, *phu* 13-14 x *chc* PI 275136, *'phu'* ID5 x *chc* 55-1, and *'phu'* ID5 x *phu* 13-14, showed a rapid collapse during the period of August 5 to 13, 1994 in the greenhouse. All were among the slowest families to develop in the greenhouse and only some of the first family flowered. Other than slow growth, especially of the latter two families, plants appeared healthy in all three families until August 5 when plants appeared waterlogged. Gradually until August 13 plants died with nearly total mortality in the latter two families and approximately 50% mortality in the first.

Tables 3-7, 3-8, and 3-9 present analyses of variance for CPB damage at four, eight, and 12 weeks post-transplant, respectively. Treatment was a significant source of variation at week 4 **(Table 3-7)** but not at weeks 8 and 12 **(Table 3-8** and **3-9)**. Significance of block within treatment at 8 and 12 weeks may reflect irregular distribution of CPB individuals in natural infestations at week four. As illustrated in **Table 3-10,** unsprayed blocks suffered more CPB damage. **Table 3-11** shows CPB damage over time in the various types of hybrids and control populations. Interestingly, intraspecific *S. phureja* hybrids suffered decreasing damage over the course of the growing season whereas control *S. phureja* clones suffered increased damage over time. Furthermore, hybrids derived from leptinidine/acetylleptinidine synthesizing *S. chacoense* accessions (those followed by **alk** in **Table 3-11)** did not necessarily suffer less damage than corresponding hybrids without this capability, although differences between these groups were not statistically different in the three weeks studied.

Tables 3-12, 3-13, and 3-14 show the analysis of variance for tuber set per plant, total tuber weight per plant, and average tuber weight, respectively. The type of hybrid and family nested within type were statistically significant for tuber number per plant **(Table 3-12)**, while treatment, block within treatment, type of hybrid, and family nested within type of hybrid were all significant for total tuber weight **(Table 3-13)** and average tuber weight **(Table 3-14)**. Spraying for CPB had no significant effect on tuber set per plant, but significantly increased total tuber weight per plant and average tuber weight **(Table 3-15)**. As expected, *S. tuberosum* cultivar checks

had the highest total tuber weight per plant and average weight per tuber **(Table 3-16)**. As can be seen from this table, however, PC and CP hybrids had higher total weights per plant than the *S. phureja* populations, although the differences were not significant. CP hybrids were significantly lower than the *S. phureja* control and were not statistically higher than the PP hybrids.

Discussion

True potato seed (TPS) families, as illustrated by the results presented here, are extremely variable due to sexual recombination, as has been noted repeatedly in the past (Arndt and Peloquin 1990; Brown and Huaman 1984; Concilio and Peloquin 1987; Engels et al. 1993a, 1993b, 1993c; Fernandez et al. 1988; Jellis and Richardson 1987; Kidane-Mariam et al. 1984, 1985; Macaso-Khwaja et al. 1983; Pallais 1987; Shonnard and Peloquin 1991a, 1991b; Wiersema 1986; Wiersema and Cabello 1986; Zehnder and Evanylo 1988). TPS generated from crossing may be used to develop virus free stock from parents contaminated by many viruses, such as potato virus S (PVS) (deBokx 1972) and potato virus T (PVT) (Jones et al. 1982). The seedling variation observed in our study was desirable to observe variation in vigor and leptine glycoalkaloids.

The instability observed in some of the families is characteristic of crosses observed previously in hybrids between these two species (Bani-Aameur et al. 1991, 1993). A reported cause for such hybrid breakdown is conflict between cytoplasms if

one of the hybrid species is native to Mexico and one is native to South America (Abdalla and Ramanna 1971). This is not the case for our hybrid populations as both parental species are South American in distribution. Differences in endosperm balance number (EBN) has been illustrated to make some *Solanum* hybrid combinations impossible or unstable, but this is also not the case for our hybrids as both possess an equivalent EBN number (Brown 1988; Brown and Adiwilaga 1991; Noy and Hanneman 1991). Chromosomal elimination has also been shown to cause breakdown in some *Solanum* interspecific hybrids (Ramanna and Hermsen 1971). We have not performed karyotypic analysis of our visibly unstable hybrids and cannot, therefore, ascribe the instability observed in our hybrids to this mechanism. Instability within other attempted interspecific *Solanum* hybridizations has been attributed to so-called 'seed set genes' within the two respective genomes, whose products interfere with each other to prevent set of viable seed during the development process (Summers and Grun 1981). This may, in addition to karyotypic study, offer an avenue of study for these hybrids.

From the results observed here, spraying for CPB resulted in increased tuber weight per plant and average tuber weight. This increase likely resulted from deterred feeding by CPB. Aside from *S. phureja* control 13-14, which was killed by defoliation in both blocks it was present in, and several hybrid plants which did not tuberize, most hybrids, even in the unsprayed blocks, suffered much less damage from CPB feeding later in the season, as compared to earlier in the season. Lower yields of the hybrid families, compared to *S. tuberosum*, was mostly due to the genetic nature of

the hybrids in that they were comprised essentially of unselected germplasm of a wild and weedy nature. The agronomic characters of the PC and CP hybrid families that were selected for field evaluation were intermediate to the *S. phureja* and *S. chacoense* parents, as expected. These groups showed CPB resistance similar to *phu* controls at 4 and 8 weeks and were intermediate between *S. chacoense* and *S. phureja* at 12 weeks. Thus, further selection and hybridization, aided by observations of high fertility of the hybrid families, particularly in the PC families 13-14 x 275136 and 13-14 x 55-1, should facilitate production of higher quality exotic germplasm.

Of special note is the observation that hybrids containing a leptine-synthesizing *S. chacoense* clone in their parentage were not necessarily more resistant than hybrids with other *S. chacoense* accessions. Several accessions utilized in this study did not synthesize leptines but were selected based on reported CPB resistance (Hanneman and Bamberg 1986). This allows the possibility of introgressing multiple forms of CPB resistance into cultivated potato from *S. chacoense*.

Lastly, spraying for CPB control seemed to decrease in effectiveness during the growing season. While damage generally declined during the season, the differences between sprayed and unsprayed blocks became insignificant during the last third to half of the season. This is possibly due to selection within the initial CPB population for individuals resistant to the pesticides applied. This is consistent with the observation that CPB populations are diverse in terms of resistance to pesticides. The biochemical, metabolic, and/or physiological mechanisms contributing to this observation are unknown and should prove useful to later breeding work in this area.

Many PC and CP hybrid families exhibited hybrid breakdown. Some families were uniformly vigorous, some were uniformly weak, and some showed segregation for vigor, with vigorous individuals, weak individuals, and individuals that died rapidly after four months of vigorous growth. This variation illustrates the importance of maintaining a broad genetic background in this, and all, breeding programs.

In summary, interspecific hybrids between *S. chacoense* and *S. phureja* can be developed that possess sufficient Colorado potato beetle resistance, vigor, and fertility to be further utilized in breeding projects. The instability observed in earlier studies of hybrids between these two species was again observed here and appears to be a incompatible interaction between selected clones representing either. The levels of vigor observed in this study varied among hybrid families from lack of germination to uniformly weak families to uniformly vigorous families. It was hypothesized initially in this study that previous selection of high alkaloid synthesizing *S. chacoense* clones exclusively for hybridization with *S. phureja* may have limited the success of hybridizations due to some unsuitable characters linked to high leptine alkaloid synthesis in *S. chacoense*. This does not seem to be the case in that most interspecific hybrid families showed some degree of hybrid depression. Also, the non alkaloid resistance mechanism present in some *S. chacoense* clones seems to convey resistance as well as the alkaloid resistance mechanism, opening the possibility for introgression of multiple resistance mechanisms into *S. tuberosum*. This resistance should be more stable than either alone and be beneficial to genetic control of CPB. Some vigorous seedlings selected in the field for CPB resistance also possess good tuberization

qualities and fertility, indicating that advanced germplasm development should be possible, with eventual hybridization to 2x or 4x *S. tuberosum*.

Literature Cited

Abdalla MF and Ramanna MS (1971) Male sterility in *Solanum polytrichon* X *S. phureja* hybrid, caused by plasmon-genic interaction and its significance. Euphytica 20: 482-489.

Arndt GC and Peloquin SJ (1990) The identification and evaluation of hybrid plants among open pollinated true seed families. Amer. Potato J. 67: 393-404.

Bani-Aameur F, Lauer FI, and Veilleux RE (1993) Enhancement of diploid *Solanum chacoense* Bitt. using adapted clones of *Solanum phureja* Juz. & Buk. Euphytica 68: 169-179.

Bani-Aameur F, Lauer FI, Veilleux RE, and Hilali A (1991) Genomic composition of 4x-2x potato hybrids: influence of *Solanum chacoense*. Genome 34: 413-420.

Bernardello LM, Heiser CB, and Piazzano M (1994) Karyotypic studies in *Solanum* section *Lasiocarpa* (Solanaceae). Amer. J. Bot. 81: 95-103.

Birhman RK, Rivard SR, and Cappadocia M (1994) Restriction fragment length polymorphism analysis of anther-culture-derived *Solanum chacoense*. HortScience 29: 206-208.

Bishop BA and Grafius E (1991) An on-farm insecticide resistance test kit for Colorado potato beetle (Coleoptera: Chrysomelidae). Amer. Potato J. 68: 53-64.

Bonierbale MW, Plastid RL, and Tanksley SD (1993) A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. Theor. Appl. Genet. 86: 481-491.

Brown CR (1988) Characteristics of 2n pollen producing triploid hybrids between *Solanum stoloniferum* and cultivated dihaploid potatoes. Amer. Potato J. 65: 75-84.

Brown CR and Adiwilaga K (1991) Use of rescue pollinations to make a complex interspecific cross in potato. Amer. Potato J. 68: 813-820.

Brown CR and Huaman KD (1984) Estimation of outcrossing rates in Andigena cultivars: implications in breeding TPS cultivars. In: 6th symposium of the International Society for Tropical Root Crops, 1983. International Potato Center. Lima, Peru pp. 473-480.

Brown CR, Mojtahedi H, and Santo GS (1991) Resistance to Columbia root-knot nematode in *Solanum* spp. and in hybrids of *S. hougasii* with tetraploid cultivated potato. Amer. Potato J. 68: 445-452.

Cantelo WW, Douglass LW, Sanford LL, Sinden SL, and Deahl KL (1987) Measuring resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in potato. J. Entomol. Sci. 22: 245-252.

Cappadocia M and Ahmim C (1988) Comparison of two culture methods for the production of haploids by anther culture in *Solanum chacoense*. Can. J. Bot. 66: 1003-1005.

Cappadocia M, Cheng DSK, and Ludlum-Simonette R (1986) Self-compatibility in doubled haploids and their F_1 hybrids, regenerated via anther culture in self-incompatible *Solanum chacoense* Bitt. Theor. Appl. Genet. 72: 66-69.

Cappadocia M, Cheng DSK, and Ludlum-Simonette R (1984) Plant regeneration from in vitro culture of anthers of *Solanum chacoense* Bitt. and interspecific diploid hybrids of *S. tuberosum* L. x *S. chacoense* Bitt. Theor. Appl. Genet. 69: 139-143.

Cardi T, D'Ambrosio F, Consoli D, Puite KJ, and Ramulu KS (1993) Production of somatic hybrids between frost-tolerant *Solanum commersonii* and *S. tuberosum*: characterization of hybrid plants. Theor. Appl. Genet. 87: 193-200.

Carter CD (1987) Screening *Solanum* germplasm for resistance to Colorado potato beetle. Amer. Potato J. 64: 563-568.

Chase SS (1963) Analytic breeding in *Solanum tuberosum* L. -- a scheme utilizing parthenotes and other diploid stocks. Can. J. Genet. Cytol. 5: 359-363.

Concilio L and Peloquin SJ (1987) Tuber yield of true potato seed families from different breeding schemes. Amer. Potato J. 64: 81-85.

Correll DS (1962) The Potato and its Wild Relatives. Texas Research Foundation. Renner, Texas 606 pp.

Costa SD and Gaugler R (1989) Influence of *Solanum* host plants on Colorado potato beetle (Coleoptera: Chrysomelidae) susceptibility to the entomopathogen *Beauveria bassiana*. Environ. Entomol. 18: 531-536.

Cutler HC and Whitaker TW (1969) A new species of *Cucurbita* from Ecuador. Ann. Missouri Bot. Gard. 55: 392-396.

D'Antonio VL and McHale NA (1988) Effect of storage temperature and extraction methods on dormancy and germination of true potato seed. Amer. Potato J. 65: 573-581.

D'Arcy WG (1991) The *Solanaceae* since 1976, with a review of its biogeography. In: Hawkes JG, Lester RN, Nee M, and Estrada N (Eds.) Solanaceae III: taxonomy, chemistry, evolution. Royal Botanic Gardens. Kew, England. pp. 75-137.

D'Arcy WG (1979) The classification of the Solanaceae. In: Hawkes JG, Lester RN, and Skelding AD (Eds.) The biology and taxonomy of the Solanaceae. Academic Press. London, England. pp. 3-47.

Deahl KL and Sinden SL (1987) A technique for rapid detection of leptine glycoalkaloids in potato foliage. Amer. Potato J. 64: 285-290.

Deahl KL, Cantelo WW, Sinden SL, and Sanford LL (1991) The effect of light intensity on Colorado potato beetle resistance and foliar glycoalkaloid concentration of four *Solanum chacoense* clones. Amer. Potato J. 68: 659-666.

Deahl KL, Sinden SL, and Young RJ (1993) Evaluation of wild tuber-bearing *Solanum* accessions for foliar glycoalkaloid level and composition. Amer. Potato J. 70: 61-69.

DeBokx JA and van der Want JPH (Ed.) (1972) Viruses of Seed Potatoes and Seed Potato Production. Centre for Agricultural Publishing and Documentation. Wageningen. 259 pp.

Engels C, El Bedewy R, and Sattelmacher B (1993a) Effects of weight and planting density of tubers derived from true potato seed on growth and yield of potato crops in Egypt. 1. Sprout growth, field emergence and haulm development. Field Crops Res. 35: 159-170.

Engels C, El Bedewy R, and Sattelmacher B (1993b) Effects of weight and planting density of tubers derived from true potato seed on growth and yield of potato crops in Egypt. 2. Tuber yield and tuber size. Field Crops Res. 35: 171-182.

Engels C, El Bedewy R, and Sattelmacher B (1993c) Seed tuber production from true potato seed (TPS) in Egypt and the influence of environmental conditions in different growing periods. Potato Res. 36: 195-203.

Ewing EE (1981) Heat stress and the tuberization response. Amer. Potato J. 58: 31-49.

Fernandez BB, Tumapon AS, Duna LA, Balanay NM, Kloos JP, and Vander Zaag P (1988) On-farm evaluation of true potato seed in the Philippines. Amer. Potato J. 65: 457-461.

Ferro DN and Boiteau G (1993) Management of insect pests. In: Rowe RC (Ed.) (1993) Potato Health Management. APS Press. St. Paul. pp. 103-108.

Forgash AJ (1985) Insecticide resistance in the Colorado potato beetle. In: Ferro DN and Voss RH (Eds.) Proc. Symp. Colorado potato beetle, 17th Congress of Entomology. pp. 33-52.

Grant V (1966) Block inheritance of viability genes in plant species. Amer. Nat. 100: 591-601.

Grun P and Chu L (1978) Development of plants from protoplasts of *Solanum* (Solanaceae). Amer. J. Bot. 65: 538-543.

Hanneman Jr. RE and Bamberg JB (1986) Inventory of Tuber-Bearing *Solanum* species. University of Wisconsin, Madison. Bulletin 533.

Harland SC (1936) The genetical conception of a species. Biol. Rev. 11: 83-112.

Harrison GD (1987) Host-plant discrimination and evolution of feeding preference in the Colorado potato beetle *Leptinotarsa decemlineata*. Physiol. Entom. 12: 407-415.

Hawkes JG (1963) A revision of the tuber-bearing Solanums, 2nd ed. Scottish Plant Breeding Station Record 1963. Pentlandfield, Scotland. pp. 76-181.

Haynes FL (1972) The use of cultivated *Solanum* species in potato breeding. In: French ER (Ed.) Prospects for the potato in the developing world: an international symposium on key problems and potentials for greater use of the potato in the developing world, Lima, Peru., July 17-29, 1972. International Potato Center, Lima, p. 62-73.

Jacobsen E, Malver R, Huigen DJ, Bergervoet JEM, and Ramanna MS (1993) Isolation and characterisation of somatic hybrids of diploid *Solanum tuberosum* and *Solanum brevidens* and the use of amylose-free starch mutation for detection of introgression. Euphytica 69: 191-201.

Jellis GJ and Richardson DE (Eds.) (1987) The production of new potato varieties: technological advances. Cambridge University Press New York, New York ch 3.

Jones RAC (1982) Tests for transmission of four potato viruses through potato true seed. Ann. Appl. Biol. 100: 315-320.

Kidane-Mariam HM, Mendoza HA, and Wissar RO (1985) Performances of true potato seed families derived from intermating tetraploid parental lines. Amer. Potato J. 62: 643-652.

Kidane-Mariam HM, Mendoza HA, and Wissar RO (1984) Intervarietal hybridization for potato production from true potato seed. In: 6th Symp. Internat. Soc. for Tropical Root Crops, 1983. International Potato Center. Lima, Peru pp 487-492.

Lu W and Logan P (1993) Induction of feeding on potato in Mexican *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Environ. Entomol. 22: 759-765.

Macaso-Khwaja AC and Peloquin SJ (1983) Tuber yields of families from open pollinated and hybrid true potato seed. Amer. Potato J. 60: 645-651.

McCollum GD and Sinden SL (1979) Inheritance study of tuber glycoalkaloids in a wild potato, *Solanum chacoense* Bitter. Amer. Potato J. 56: 95-113.

Melville AA, Storch RH, Bushway RJ, and Alford AR (1985) Growth and feeding of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), fed foliage of three *Solanum* species. Maine Ag. Exp. St. Tech Bull. 115.

Mitchell BK (1987) Interactions of alkaloids with galeal chemosensory cells of Colorado potato beetle. J. Chem. Ecol. 13: 2009-2022.

Mooney JJ and Jansky SH (1990) Development of insect resistant interspecific *Solanum* hybrids. Proc. N. D. Acad. Sci. 44: 73. (Abs.)

Nowak J and Colborne D (1989) *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. Amer. Potato. J. 66: 35-45.

Novy RG and Hannman Jr. RE (1991) Hybridization between Gp. Tuberosum haploids and 1EBN wild potato species. Amer. Potato J. 68: 151-169.

Osman SF; Sinden SL; Deahl KL; and Moreau R (1987) The metabolism of solanidine by microsomal fractions from *Solanum chacoense*. Phytochemistry 26: 3163-3165.

Osman SF; Herb SF; Fitzpatrick TJ; and Sinden SL (1976) Commersonine. A new glycoalkaloid from two *Solanum* species. Phytochemistry 15: 1065-1067.

Pallais N (1987) True potato seed quality. Theor. Appl. Genet. 73: 784-792.

Percival GC, Harrison JAC, and Dixon GR (1993) The influence of temperature on light enhanced glycoalkaloid synthesis in potato. Ann. Appl. Biol. 123: 141-153.

Perlak FJ, Stone TB, Muskopf YM, Petersen LJ, Parker GB, McPherson SA, Wyman J, Love S, Reed G, Biever D, and Fischoff DA (1993) Genetically improved potatoes: protection from damage by Colorado potato beetle. Plant Mol. Biol. 22: 313-321.

Ramanna MS and Hermsen JG (1971) Somatic chromosome elimination and meiotic chromosome pairing in the triple hybrid 6x-(*Solanum acaule* X *S. bulbocastanum*) X 2x-*S. phureja*. Euphytica 20: 470-481.

Reynolds MP and Ewing EE (1989) Heat tolerance in tuber bearing *Solanum* species: a protocol for screening. Amer. Potato J. 66: 63-73.

Rivard SR, Cappadocia M, Vincent G, Brisson N, and Landry BS (1989) Restriction fragment length fragment polymorphism (RFLP) analyses of plants produced by in vitro anther culture of *Solanum chacoense* Bitt. Theor. Appl. Genet. 78: 49-56.

Rowe RC (1993) Potato Health Management: a holistic approach. In: Rowe RC (Ed.) Potato Health Management. APS Press. St. Paul. pp. 1-10.

Sanford LL, Deahl KL, and Sinden SL (1994) Glycoalkaloid content in foliage of hybrid and backcross populations from a *Solanum tuberosum* X *S. chacoense* cross. Amer. Potato J. 71: 225-235.

Sanford LL, Deahl KL, Sinden SL, and Ladd Jr. TL (1992) Glycoalkaloid contents in tubers from *Solanum tuberosum* populations selected for potato leafhopper resistance. Amer. Potato J. 69: 693-703.

Sanford LL and Ladd Jr. TL (1992) Performance of populations derived by selection for resistance to potato leafhoppers in a 4x *Solanum tuberosum* X 2x *Solanum chacoense* cross. Amer. Potato J. 69: 391-400.

Sax K (1933) Species hybrids in *Platanus* and *Campsis*. J. Arnold Arbor. 14: 274-278.

SAS Institute Inc. (1985) SAS user's guide: statistics. SAS Institute Inc., Cary, NC.

Shonnard GC and Peloquin SJ (1991a) Performance of true potato seed families. I. Effect of level of inbreeding. Pot. Res. 34: 397-407.

Shonnard GC and Peloquin SJ (1991b) Performance of true potato seed families. II. Comparison of transplants vs. seedling tubers. Pot. Res. 34: 409-418.

Sinden SL, Sanford LL, and Deahl KL (1986a) Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter. J. Agric. Food Chem. 34: 372-377.

Sinden SL, Sanford LL, Cantelo WW, and Deahl, KL (1986b) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in *Solanum chacoense*. Environ. Entomol. 15: 1057-1062.

Sinden SL, Sanford LL, and Osman SF (1980) Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* Bitter. Amer. Potato J. 57: 331-343.

Spooner DM, Anderson GJ, and Jansen RK (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Amer. J. Bot. 80: 676-688.

Stebbins Jr. GL (1945) The cytological analysis of species hybrids. II. Bot. Rev. 11: 463-486.

Stephens SG (1950) The internal mechanism of speciation in *Gossypium*. Bot. Rev. 16: 115-149.

Stephens SG (1949) The cytogenetics of speciation in *Gossypium*. I Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics 34: 627-637.

Summers D and Grun P (1981) Reproductive isolation barriers to gene exchange between *Solanum chacoense* and *S. commersonii* (Solanaceae). Amer. J. Bot. 68: 1240-1248.

Waara S, Wallin A, and Eriksson T (1991) Production and analysis of intraspecific somatic hybrids of potato (*Solanum tuberosum* L.). Plant Sci. 75: 107-115.

Weeden NF and Robinson RW (1986) Allozyme segregation ratios in the interspecific cross *Cucurbita maxima* X *C. ecuadorensis* suggest that hybrid breakdown is not caused by minor alterations in chromosome structure. Genetics 114: 593-609.

Wierenga JM and Hollingworth RM (1993) Inhibition of altered acetylcholinesterases from insecticide-resistant Colorado potato beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86: 673-679.

Wiersema SG (1986) The effect of density on tuber yield in plants grown from true potato seed in seed beds during two contrasting seasons. Amer. Potato J. 63: 465-472.

Wiersema SG and Cabello R (1986) Comparative performance of different-sized seed tubers derived from true potato seed. Amer. Potato J. 63: 241-249.

Xu B, Mu J, Nevins DL, Grun P, and Kao T (1990) Cloning and sequencing of cDNA's encoding two self-incompatibility associated proteins in *Solanum chacoense*. Mol. Gen. Genet. 224: 341-346.

Zehnder GW and Evanylo GK (1988) Influence of Colorado potato beetle sample counts and plant defoliation on potato tuber production. Amer. Potato J. 65: 725-736.

Table 3-1a. Pollination efficiency in terms of fruit set per pollination.

		S. phureja (♂)			*S. chacoense* (♂)	
		1-3	13-14	PP5	55-1	80-1
S. phureja (♀)	1-3	---*	---	---	0/68	0/73
	13-14	---	---	---	5/82	0/49
	PP5	---	---	---	0/67	0/82
	ID4	0/77	0/71	0/84	18/82	0/85
	ID5	0/72	3/64	0/66	14/74	0/76
	ID8	0/62	3/63	5/64	32/92	0/76
S. chacoense (♀)	55-1	0/68	0/74	0/79	---	---
	80-1	0/77	10/67	0/74	---	---
	175419	---	---	---	---	---
	414143	---	---	---	---	---
	472810	1/58	1/59	2/130	---	---

Table 3-1a. (cont) Pollination efficiency in terms of fruit set per pollination.

		S. chacoense (♂)					
		133123	175419	209411	275136	414143	472810
S. phureja (♀)	1-3	0/74	0/77	0/62	0/61	1/63	0/72
	13-14	0/95	4/81	0/72	3/64	1/60	4/76
	PP5	0/95	0/80	0/68	0/73	0/76	0/74
	ID4	0/72	0/68	0/74	0/82	0/76	0/71
	ID5	0/87	3/82	0/71	0/73	0/76	0/75
	ID8	3/56	13/82	3/87	13/62	6/64	0/72
S. chacoense (♀)	55-1	4/62	4/61	6/62	---	---	---
	80-1	4/76	5/64	3/66	---	---	---
	175419	1/62	---	---	---	---	---
	414143	6/66	7/71	2/68	---	---	---
	472810	---	---	---	---	---	---

*note: --- indicates no pollinations were attempted.

Table 3-1b. Seed production per fruit.

		S. phureja (♂)			*S. chacoense* (♂)	
		1-3	13-14	PP5	55-1	80-1
S. phureja (♀)	1-3	---	---	---	0	0
	13-14	---	---	---	60.6	0
	PP5	---	---	---	0	0
	ID4	---	---	---	147.9	0
	ID5	0	37.2	0	74.3	0
	ID8	0	46.3	111.8	158.9	0
S. chacoense (♀)	55-1	0	0	0	---	---
	80-1	0	46.3	0	---	---
	175419	---	---	---	---	---
	414143	---	---	---	---	---
	472810	43.0	54.0	37.5	---	---

Table 3-1b. (cont) Seed production per fruit.

		S. chacoense (♂)					
		133123	175419	209411	275136	414143	472810
S. phureja (♀)	1-3	0	0	0	0	29.0	0
	13-14	0	47.3	0	51.3	5.0	83.8
	PP5	0	0	0	0	0	0
	ID4	0	0	0	0	0	0
	ID5	0	45.0	0	0	0	0
	ID8	200.1	415.6	124.0	115.7	153.7	0
S. chacoense (♀)	55-1	86.3	93.8	33.8	---	---	---
	80-1	49.5	37.0	100.0	---	---	---
	175419	87.0	---	---	---	---	---
	414143	111.8	116.0	44.0	---	---	---
	472810	---	---	---	---	---	---

*note: --- indicates no seeds were obtained because the cross was not attempted. 0 indicated no seeds were obtained because the cross was not successful.

Table 3-2. Pollination efficiency between and among groups of families.

family type*	number of hybrid combinations attempted	pollinations performed	total number of fruit produced	pollination efficiency (fruit/ pollination)	pollination efficiency within families producing fruit ***
PP	10	623	11	0.018	0.058 a
CC	10	658	42	0.064	0.069 a
PC	48	3527	123	0.035	0.106 a
CP	9	686	14	0.020	0.061 a
total	77	5494	190	0.043	0.086

*note: PP indicates *S. phureja* X *S. phureja* hybrid families; CC indicates *S. chacoense* X *S. chacoense* hybrid families; PC indicates *S. phureja* X *S. chacoense* hybrid families; and CP indicates *S. chacoense* X *S. phureja* hybrid families. *** Means are not statistically different using SNK ($P<0.05$).

Table 3-3. Seeds used for greenhouse and field evaluation of plants.

		S. phureja (♂)			*S. chacoense* (♂)	
		1-3	13-14	PP5	55-1	80-1
S. phureja (♀)	13-14	---	---	---	64	0
	ID4	0	0	0	64	0
	ID5	0	64	0	64	0
	ID8	0	64	64	64	0
S. chacoense (♀)	80-1	0	64	0	---	---
	472810	43	54	0	---	---

		S. chacoense (♂)					
		133123	175419	209411	275136	414143	472810
S. phureja (♀)	1-3	0	0	0	0	29	0
	13-14	0	64	0	64	64	64
	ID5	0	64	0	0	0	0
	ID8	64	64	64	64	64	0
S. chacoense (♀)	55-1	64	64	64	---	---	---
	80-1	64	64	49	---	---	---
	414143	64	64	64	---	---	---

--- indicates no seed were used in this study because the cross was not attempted. 0 indicates no seed were used in this study because no seed were obtained from crossing.

Table 3-4. Average percentage of surviving plants at each stage in each of the four groups of seedling families. Numbers in parentheses represent the reduction in the percentage of potential plants from the previous stage.

family type*	n	average germination %	average % alive at 6 weeks
PP	3	58.9 (+/-12.2) a**	28.1 (30.8)
CC	9	53.5 (+/-31.7) a	42.0 (11.5)
PC	15	47.0 (+/-35.3) a	34.0 (44.3)
CP	3	61.5 (+/-11.3) a	29.1 (32.4)
total	30	51.6	33.3 (29.8)

*note: PP indicates *S. phureja* X *S. phureja* hybrid families; CC indicates *S. chacoense* X *S. chacoense* hybrid families; PC indicates *S. phureja* X *S. chacoense* hybrid families; and CP indicates *S. chacoense* X *S. phureja* hybrid families. **means within the same column followed by the same letter are not statistically different using SNK ($P<0.05$). Numbers following germination percentages in parentheses are +/- one standard deviation.

Table 3-5. Survival among four groups of families under field conditions.

family type*	n	number of seedlings transplanted	survival after 2 weeks	survival after 4 weeks	survival at full flowering
PP	2	24	20	19	16
CC	5	60	57	51	49
PC	9	108	104	98	89
CP	2	24	23	22	18
total	18	216	204	190	172

*note: see **Table 3-2** for description of family types.

Table 3-6. Survival among four groups of hybrid families under greenhouse conditions.

family type*	n	number of seedlings transplanted	survival after 2 weeks after transplant	survival after 4 weeks after transplant
PP	3	43	43	43
CC	7	182	181	177
PC	12	235	232	231
CP	3	24	23	23
total	25	484	479	474

*note: see **Table 3-2** for description of family types.

Table 3-7. Analysis of variance for Colorado potato beetle (*Leptinotarsa decemlineata* Say) damage among four types of hybrid families and three control populations four weeks after transplant.

source	df	Mean square	F value	Pr > F
treatment	1	9.800	16.88	0.0002
block (treatment)	2	1.63	2.81	0.0700
type of hybrid	7	1.65	12.34	0.0001
family (type of hybrid)	14	0.49	0.84	0.6239
treatment*type of hybrid	6	0.95	1.63	0.1583

Table 3-8. Analysis of variance for Colorado potato beetle (*Leptinotarsa decemlineata* Say) damage among four types of hybrid families and three control populations eight weeks after transplant.

source	df	Mean square	F value	Pr > F
treatment	1	0.03	0.06	0.8045
block (treatment)	2	1.43	3.15	0.0518
type of hybrid	7	3.05	6.72	0.0001
family (type of hybrid)	14	0.70	1.55	0.1281
treatment*type of hybrid	6	0.21	0.47	0.8292

Table 3-9. Analysis of variance for Colorado potato beetle (*Leptinotarsa decemlineata* Say) damage among four types of hybrid families and three control populations twelve weeks after transplant.

source	df	Mean square	F value	Pr > F
treatment	1	0.003	0.01	0.9413
block (treatment)	2	2.27	3.97	0.0252
type of hybrid	7	9.41	16.50	0.0001
family (type of hybrid)	14	0.46	0.81	0.6559
treatment*type of hybrid	6	0.32	0.56	0.7631

Table 3-10. Colorado potato beetle (*Leptinotarsa decemlineata* Say) damage ratings for blocks either sprayed for control or not. The rating scale extends from 0 (no damage) to 5 (total defoliation and eventual death).

spray treatment	4 weeks after transplant	8 weeks after transplant	12 weeks after transplant
unsprayed	2.4 a*	1.8 a	1.5 a
sprayed	1.7 b	1.8 a	1.5 a

*note: means within a column followed by the same letter are not statistically different (LSD P<0.05).

Table 3-11. Colorado potato beetle (*Leptinotarsa decemlineata* Say) damage ratings for hybrid families at 4, 8, and 12 weeks after transplant. The rating scale extends from 0 (no damage) to 5 (total defoliation and eventual death).

genotypic description	4 weeks after transplant	8 weeks after transplant	12 weeks after transplant
PP hybrids	3.3 a*	2.7 a	1.7 b
CP hybrids (alk)**	2.8 ab	1.8 bc	2.1 b
PC hybrids	2.5 b	2.2 ab	1.7 b
phu control	2.3 b	2.1 ab	4.3 a
PC hybrids (alk)	2.2 b	1.8 bc	1.9 b
CC hybrids	1.3 c	1.3 cd	0.5 c
CC hybrids (alk)	0.8 c	1.0 cd	0.5 c
chc control	0.3 d	0.6 d	0.0 c

*note: means within a column followed by the same letter are not statistically different (LSD P<0.05). **Genotypes followed by **(alk)** are hybrids with a parental *S. chacoense* accession that synthesized leptinidine and acetylleptinidine.

Table 3-12. Analysis of variance for tuber set per plant among four hybrid types and three control populations.

source	df	Mean square	F value	Pr > F
treatment	1	134.39	2.15	0.1438
block (treatment)	2	31.95	0.51	0.6002
type of hybrid	6	200.94	3.22	0.0048
family (type of hybrid)	18	258.87	4.15	0.0001
treatment*type of hybrid	4	37.59	0.60	0.6617

Table 3-13. Analysis of variance for total tuber weight per plant among four hybrid types and three control populations.

source	df	Mean square	F value	Pr > F
treatment	1	78024.8	11.45	0.0008
block (treatment)	2	22676.3	3.33	0.0377
type of hybrid	6	205477.0	30.15	0.0001
family (type of hybrid)	18	153841.0	22.58	0.0001
treatment*type of hybrid	4	0.1	0.12	0.9740

Table 3-14. Analysis of variance for average tuber weight among four hybrid types and three control populations.

source	df	Mean square	F value	Pr > F
treatment	1	362.54	12.84	0.0004
block (treatment)	2	448.66	15.89	0.0001
type of hybrid	6	2749.90	97.37	0.0001
family (type of hybrid)	17	1054.22	37.33	0.0001
treatment*type of hybrid	4	20.133	0.71	0.5846

Table 3-15. Mean tuber number per plant, total tuber weight per plant, and average tuber weight in blocks sprayed or not sprayed for CPB.

spray treatment	tuber set per plant	total tuber weight	average tuber
sprayed	9.7 a*	86.1 a	8.7 a
unsprayed	8.3 a	50.7 b	6.0 b

* note: means within a column followed by the same letter are not statistically different (LSD P<0.05).

Table 3-16. Mean tuber number per plant, total tuber weight per plant, and average tuber weight among four family types and three control populations.

genotypic description	tuber set per plant	total tuber weight per plant (g)	average tuber weight (g)
PC hybrids	11.0 a*	78.4 b	7.0 bc
CP hybrids	10.9 a	65.9 b	5.6 c
chc control	8.7 a	25.3 b	2.9 c
PP hybrids	7.0 ab	39.5 b	5.3 c
CC hybrids	6.9 ab	24.8 b	3.3 c
tbr control	5.8 ab	409.4 a	54.7 a
phu control	4.2 b	40.8 b	11.0 b

*note: means within a column followed by the same letter are not statistically different (LSD $P<0.05$).

APPENDIX A Media

Medium 1 Liquid Anther Culture Medium (Uhrig, 1985)

MS I (macronutrients)	10 ml/l
MS II ($CaCl_2 \cdot H_2O$)	10 ml/l
MS III (Fe·EDTA)	5 ml/l
MS IV (micronutrients)	5 ml/l
myo inositol	100 mg/l
thiamine-HCl	0.4 mg/l
IAA	0.1 mg/l
BAP	2.5 mg/l
sucrose	60 g/l
activated charcoal	2.5 g/l
pH	5.8

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Uhrig T (1985) Genetic selection and liquid medium conditions improve the yield of androgenetic plants from diploid potatoes. Theor. Appl. Genet. 71: 455-460.

Murashige Skoog stock	component	concentration in media	concentration in stock
I	NH_4NO_3	1650 mg/l	82.5 g/l
	KNO_3	1900 mg/l	95.0 g/l
	KH_2PO_4	170 mg/l	8.5 g/l
	$MgSO_4 \cdot 7H_2O$	370 mg/l	18.5 g/l
II	$CaCl_2 \cdot 2H_2O$	440 mg/l	22.0 g/l
III	$Na_2 \cdot EDTA$	37.3 mg/l	3.7 g/l
	$FeSO_4 \cdot 7H_2O$	27.8 mg/l	2.8 g/l
IV	$MnSO_4 \cdot 4H_2O$	22.3 mg/l	2.23 g/l
	$ZnSO_4 \cdot 7H_2O$	10.6 mg/l	1.06 g/l
	H_3BO_3	6.2 mg/l	620 mg/l
	KI	0.83 mg/l	83.0 mg/l
	$Na_2MoO_4 \cdot 2H_2O$	0.25 mg/l	25.0 mg/l
	$CoCl_2 \cdot 6H_2O$	0.025 mg/l	2.5 mg/l
	$CuSO_4 \cdot 5H_2O$	0.025 mg/l	2.5 mg/l
V	glycine	2.0 mg/l	200 mg/l
	nicotinic acid	0.5 mg/l	50 mg/l
	pyridoxine-HCl	0.5 mg/l	50 mg/l
	thiamine-HCl	0.1 mg/l	10 mg/l

Medium 2 Embryo Regeneration Medium modified embryo media-V-I (Gamborg et al., 1968)

B5 media and minimal inorganic salts	3.2 g/l (Sigma Chem. Co., St. Louis, MO)
MS II ($CaCl_2 \cdot H_2O$)	34 ml/l
$CaHPO_4$	50 mg/l
NH_4NO_3	250 mg/l
GA_3	0.1 mg/l (filter sterilized)
sucrose	10 g/l
agarose	6 g/l (Sigma type III-A: high EEO, Sigma Chem. Co., St. Louis, MO)
pH	5.6

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Gamborg OL, Miller RA, and Ojimal L (1968) Nutrient requirements of suspension cultures of soybean root cells. Exptl. Cell Res. 50: 151-158.

Medium 3 Basal MS Medium (Murashige and Skoog, 1962)

MS I (macronutrients)	20 ml/l
MS II ($CaCl_2 \cdot H_2O$)	20 ml/l
MS III (Fe·EDTA)	10 ml/l
MS IV (micronutrients)	10 ml/l
MS V (vitamins)	10 ml/l
myo-inositol	100 mg/l
casein hydrolysate	100 mg/l (13% N by weight, Sigma Chem. Co., St. Louis, MO)
sucrose	30 g/l
agar	7 g/l
pH	5.8

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Medium 4 Leaf Disc Regeneration Hormone Pulse Medium (Hulme et al., 1992)

MS I (macronutrients)	20 ml/l
MS II ($CaCl_2 \cdot H_2O$)	20 ml/l
MS III (Fe·EDTA)	10 ml/l
MS IV (micronutrients)	10 ml/l
MS V (vitamins)	10 ml/l
$CaCl_2$	147 mg/l
NH_4NO_3	80 mg/l
BAP	9.909 mg/l
NAA	10.05 mg/l
sucrose	10 g/l

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Hulme JS, Higgins ES, and Shields R (1992) An efficient genotype-independent method for regeneration of potato plants from leaf tissue. Plant Cell Tiss. Org. Cult. 31: 161-167.

Medium 5 Leaf Disc Regeneration Callus Induction Medium (Hulme et al., 1992)

MS I (macronutrients)	20 ml/l
MS II ($CaCl_2 \cdot H_2O$)	20 ml/l
MS III (Fe·EDTA)	10 ml/l
MS IV (micronutrients)	10 ml/l
MS V (vitamins)	10 ml/l
IAA	0.1752 mg/l
BAP	2.252 mg/l
mannitol	4 g/l
sucrose	1 g/l
agar	8 g/l

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Hulme JS, Higgins ES, and Shields R (1992) An efficient genotype-independent method for regeneration of potato plants from leaf tissue. Plant Cell Tiss. Org. Cult. 31: 161-167.

Medium 6 Leaf Disc Regeneration Shoot Regeneration Medium (Hulme et al., 1992)

MS I (macronutrients)	20 ml/l
MS II ($CaCl_2 \cdot H_2O$)	20 ml/l
MS III (Fe·EDTA)	10 ml/l
MS IV (micronutrients)	10 ml/l
MS V (vitamins)	10 ml/l
BAP	2.252 mg/l
GA_3	4.850 mg/l (filter sterilized)
sucrose	15 g/l
agar	8 g/l

autoclaved at 121°C and 1.1 kg/cm for 20 min.

Hulme JS, Higgins ES, and Shields R (1992) An efficient genotype-independent method for regeneration of potato plants from leaf tissue. Plant Cell Tiss. Org. Cult. 31: 161-167.

APPENDIX B Field spray schedule

date	chemical	rate
5/19/94	Ambush Dithane DF	147 g/ha 321 g/ha
6/10/94	Asana XL Bravo 720	110 g/ha 0.287 l/ha
7/5/94	Bravo 720	0.287 l/ha
7/15/94	Ambush Bravo 720	147 g/ha 0.287 l/ha
7/22/94	Ambush Bravo 720	147 g/ha 0.287 l/ha
8/2	Bravo 720	0.287 l/ha
8/11	Bravo 720 + Ridomil 81W	367 g/ha

CURRICULUM VITAE

David Harrison Wuosmaa was born on September 15, 1970 in Scotia, New York. He attended Shenendehowa High School, which he graduated from with a Regent's diploma and a Presidential Academic Fitness Award. He attended the New York State College of Agriculture and Life Sciences at Cornell University from 1988 to 1992, from which he received a BS in Plant Sciences with a specialization in Plant Breeding, Genetics, and Biometry. After a summer of employment overseeing maintenance and renovation of the Andrew Dickson White Gardens on the Cornell University Campus, David moved to Virginia Polytechnic Institute and State University to pursue a MS degree in Horticulture. His research specialized in potato breeding and genetics, and in particular the derivation of interspecific hybrids between *Solanum chacoense* and *S. phureja* to develop hybrids with resistance to Colorado potato beetle (Coleoptera, Chrysomelidae: *Leptinotarsa decemlineata*). Upon completion of this degree in September 1994 he will move to Summerville, South Carolina to work with Westvaco Corporation on somatic embryogenesis in loblolly pine (*Pinus taeda*).

David Harrison Wuosmaa