

# A Test of Taxonomic Predictivity: Resistance to the Colorado Potato Beetle in Wild Relatives of Cultivated Potato

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**ABSTRACT** Wild relatives of potato offer a tremendous germplasm resource for breeders. Because the germplasm base of potato is so broad and diverse, we have undertaken a series of studies to determine whether we can predict the distribution of valuable genes in wild *Solanum* species based on taxonomic or biogeographic data. This is the third study in the series. Resistance to defoliation by Colorado potato beetle, *Leptinotarsa decemlineata* Say, larvae was evaluated in 156 accessions of 41 wild *Solanum* species. The highest frequencies of resistant accessions were found in diploid species with an endosperm balance number of 1. In contrast to previous studies on resistance to foliar fungal pathogens, there was little variability in defoliation scores among plants within an accession and among accessions within a species, at least for the most resistant species. There was no strong association of Colorado potato beetle resistance in wild potato species to biogeographic data. Resistance was confirmed in species previously characterized by high levels of glycoalkaloids or dense glandular trichomes. However, we have identified additional species with resistance to the Colorado potato beetle. Mechanisms of resistance are being studied in these species and attempts will be made to introgress them into the cultivated potato.

**KEY WORDS** potato, *Solanum*, host resistance, Colorado potato beetle, wild species

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is a devastating insect pest in potato, *Solanum tuberosum* L., production systems worldwide. Although this defoliating insect likely originated in southern Mexico (Tower 1906), its range has expanded along with potato production regions. It is now distributed throughout North and Central America, Europe, and northern Asia, excluding China (<http://www.itis.gov>; Jolivet 2001).

Management strategies for the Colorado potato beetle rely heavily on the application of insecticides; however, this strategy has limitations, including insect resistance to chemicals, food safety concerns, and negative effects on nontarget organisms (Sinden et al. 1991). Host plant resistance offers an alternative or supplemental strategy for the control of the Colorado potato beetle. Currently, no commercially significant cultivar is resistant to this insect pest.

Although the germplasm base of potato cultivars is narrow, extensive genetic diversity is present in wild relatives. There are  $\approx 190$  wild potato species distributed from the southwestern United States to Chile (Spooner and Hijmans 2001). Species richness is high in central Mexico at 20° N, and in the southern hemi-

sphere, particularly in the Andean highlands between 8° and 20° S (Hijmans and Spooner 2001). Our study represents 14 of the 19 tuber-bearing series of Hawkes (1990), and all four clades of sect. *Petota* based on chloroplast restriction site phylogenies as summarized in Spooner and Salas (2006).

Wild *Solanum* species provide a very diverse and accessible germplasm resource (Ross 1986; Hanneman 1989; Peloquin et al. 1989; Hawkes 1990). They are found in a broad array of environments, including the high grasslands of the Andes, humid temperate mountain rain forests, mossy branches of trees, and cultivated fields. These wild species contain genes encoding numerous traits not found in commercial potato cultivars and represent an especially rich source of disease and pest resistance genes (Hanneman 1989, Spooner and Bamberg 1994, Jansky 2000).

Approximately 64% of wild *Solanum* species are diploid ( $2x = 24$ ), with most of the remaining species tetraploid ( $4x = 48$ ) or hexaploid ( $6x = 72$ ) (Hijmans et al. 2007). Many wild species cross readily with each other and the cultivated potato. However, ploidy does not always predict crossing success in potato, and species are often classified according to “effective” ploidy rather than actual ploidy. The success of interspecific crosses depends on a proper balance of genetic factors contributed by gametes to the endosperm, which if not in balance, condition endosperm death and the production of inviable seeds in endosperm balance number (EBN)-incompatible crosses (Johnston et al. 1980). *Solanum* species have

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consequently been assigned EBNs based on their ability to hybridize with each other (Hanneman 1994). In the absence of other crossing barriers, crosses between plants with matching EBN values are expected to be successful. Ploidy (EBN) combinations include 2x (1EBN), 2x (2EBN) (including most wild species), 4x (2EBN), 4x (4EBN) (including tetraploid potato cultivars), and 6x (4EBN).

Host plant resistance to the Colorado potato beetle has been reported in several wild *Solanum* relatives (Flanders et al. 1998). Most reports indicate that resistance is due to glandular trichomes or high levels of glycoalkaloids. Glandular trichomes provide effective resistance in *Solanum berthaultii* Hawkes and *Solanum tarijense* Hawkes (Dimock and Tingey 1988) and *Solanum polyadenium* Greenm. (Gibson 1976). Glycoalkaloids have been implicated as a resistance mechanism in *Solanum chacoense* Bitter (Sinden et al. 1986) and *Solanum neocardenasii* Hawkes and Hjert (Dimock et al. 1986). Resistance due to undetermined mechanisms has been reported in *Solanum pinnatisectum* Dunal (Pelletier and Tai 2001, Li et al. 2006), *Solanum capsicibaccatum* (Cárdenas) Ochoa, *Solanum jamesii* Torr, *Solanum okadae* Hawkes and Hjert, and *Solanum oplocense* Hawkes (Pelletier et al. 2001), *Solanum trifidum* Correll (Sikinyi et al. 1997, Pelletier and Tai 2001), and *Solanum agroglossum* Juz., *Solanum chomatophilum* Bitter, *Solanum paucisectum* Ochoa, and *Solanum tarnii* Hawkes and Hjert (Pelletier et al. 2007).

Although breeding efforts have been attempted, neither glycoalkaloids nor glandular trichomes have been effectively incorporated into commercial potato cultivars to improve resistance to the Colorado potato beetle. Although wild *Solanum* species are likely to contain additional sources of resistance, it is impractical to evaluate all 5,000 accessions held in the U.S. Potato Genebank. It would be useful to have a systematic approach to search for resistant genotypes, based on taxonomic or geographic information. Breeders often use taxonomic and ecogeographic data as an aid in determining species and accessions to include in resistance screens. They assume that resistance will be found in groups (such as species or plants from a geographic region) for which the trait has been observed in a representative subset of the group. This is our third study designed to test this assumption. That is, do taxonomic or ecogeographic data allow us to predict the distribution of Colorado potato beetle resistance genes in wild *Solanum* germplasm? Our first and second studies found tremendous variation within and among species for resistance to the fungal diseases white mold, (caused by *Sclerotinia sclerotiorum*) and early blight (caused by *Alternaria solani*), respectively (Jansky et al. 2006, 2008). No consistent associations were observed between white mold resistance and taxonomic series (based on an intuitive interpretation of morphological and crossing data), clade (based on a cladistic analysis of plastid DNA data), ploidy, breeding system, geographic distance, or climate parameters. Species and individual accessions with high proportions of disease resistant plants were identified, but

both often exhibited extensive variation. We concluded that taxonomic relationships and ecogeographic data cannot be reliably used to predict where additional sources of white mold or early blight resistance genes will be found. This approach has not, however, been investigated with respect to the potential for taxonomic or ecogeographic data to predict the distribution of Colorado potato beetle resistance genes in wild *Solanum*.

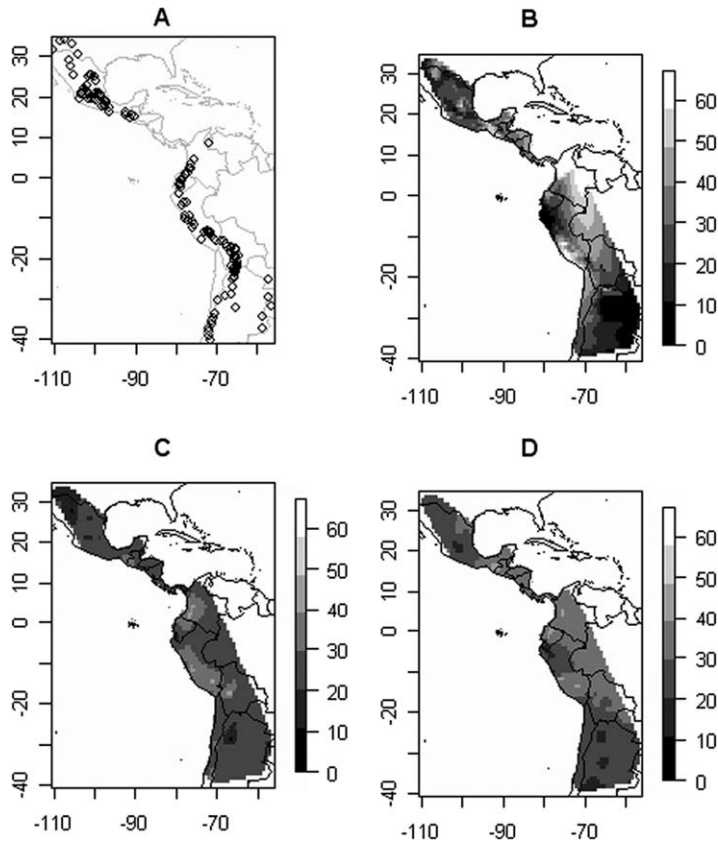
The objective of our study was to 1) determine whether Colorado potato beetle resistant phenotypes in wild species of potato germplasm are associated with taxonomic species, series, clade, ploidy, EBN, breeding system, geographic distance, or environmental factors to more efficiently choose germplasm for breeding; and 2) build on earlier tests of taxonomic prediction (Jansky et al., 2006, 2008), critically assessing the utility of taxonomic classifications and/or ecogeographical information as tools for planning and conducting effective, efficient, and comprehensive assessments of the intrinsic horticultural merit of potato genetic resources.

## Materials and Methods

**Field Trials.** On 21 April 2006, botanical seed from 41 wild *Solanum* species were sown in soil-less mix in a greenhouse in Madison, WI. For most species, four accessions were planted, resulting in 156 accessions in total. On 12 May, 24 random seedlings per accession were transplanted to individual peat pots. On 5 June, 12 random seedlings per accession were transplanted into each of two replications at the Hancock Wisconsin Agricultural Experiment Station. A randomized complete block design was used. Four tubers from 'Red Norland' potatoes also were planted after every 10 plots to provide susceptible check plants. Standard irrigation and cultural practices were used in the field, except that pesticides were not used to control Colorado potato beetle. Each plot of 12 plants was visually scored for percentage of defoliation on 2, 9, 16, and 22 August (Horton et al. 1997). The area under the defoliation progress (AUDPC) curve was calculated for each accession based on percent defoliation on the four score dates.

On 17 April 2007, seeds from the same 156 wild *Solanum* species accessions were sown in soil-less mix in a greenhouse in Madison, WI. On 9 May, 24 random seedlings were transplanted to individual peat pots. On 4 June, 12 random seedlings per accession were transplanted into each of two replications at Hancock in a randomized complete block design. As in 2006, Red Norland was included as a susceptible check. Standard cultural practices were used in the field, except that pesticides were not used to control Colorado potato beetle. Individual plants in each plot were scored for percent defoliation on 2, 9, and 16 July and 1, 13 and 21 August. The AUDPC was calculated for each accession based on mean larval defoliation scores on the six score dates.

**Statistical Analyses of Taxonomic Traits.** The General Linear model (GLM) analysis in SAS (SAS Insti-



**Fig. 1.** Comparison of results from two different strategies to examine spatial, environmental, and taxonomic associations of resistance to Colorado potato beetle. See text for explanation of strategies 1 and 2. (A) Location of accessions examined. (B–D) Interpolated data using ordinary kriging of the SAUDPDC. The legends beside B–D are in units of the SAUDPDC. B is based on raw SAUPDC; C on is based on predicted SAUDPDC by using a general linear model using six of 38 environmental variables that were determined to significantly contribute to the model; and D is uses only one of all 43 variables: species.

tute, Cary, NC) was used to analyze variation in estimated defoliation due to species, series, clade, breeding system, ploidy, and endosperm balance number. A square-root transformation was used before analysis to stabilize the variance. Means separation was carried out using the Tukey test. For multiple comparisons, a Bonferroni correction was applied. Data from each year were handled separately, because the length of the infestation (recording) period was longer in 2007 than in 2006.

**Analysis of Spatial and Environmental Variables.** The approach mainly follows that used in Jansky et al. (2006, 2008). A database with spatial and environmental data was constructed by calculating the square root of the mean Colorado potato beetle AUDPC per accession and annotating with latitude and longitude data of the source germplasm. A spatial query was conducted using localities to extract and associate monthly mean minimum and maximum monthly temperatures, mean monthly precipitation, and altitude and latitude data (38 variables in total) from a georeferenced global climate database by using DIVA-GIS software (Hijmans et al. 2001). Because wild potatoes span both sides of the equator (Fig. 1A), we

switched months for temperature classifications; that is, in the northern hemisphere, January was coded as July, February as August, and so on.

In addition to environmental variables, we examined five taxonomic variables (species, ploidy, EBN, taxonomic series, and DNA clade) for a multivariate analysis using GLM. To decide whether a model including an autocorrelation effect was significant, we compared it against a model without spatial autocorrelation by using analysis of variance (ANOVA; see Jansky et al. (2006, 2008) for explanations on the methodology and importance of spatial autocorrelation analyses.

The general linear models were explored for significance at  $P = 0.05$  for each variable. If significant, they were included in a minimal model. For visualization purposes, raw Colorado potato beetle resistance data were interpolated using a standard geographic kriging technique (Cressie 1993) as implemented in the R package (Ihaka and Gentleman 1996) by using the fields routine (Nychka 2007). The same technique was used to display the predicted values of the multivariate analyses by using geographic, environmental, and taxonomic variables as

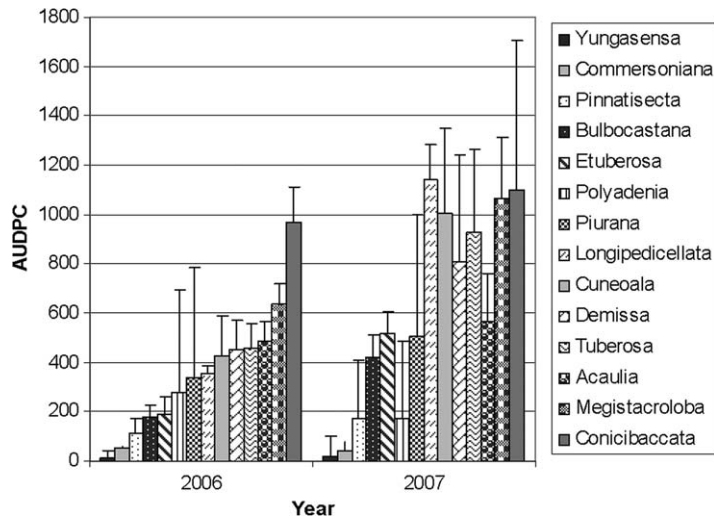


Fig. 2. Mean AUDPC scores for 14 series in sect. *Petota* in 2006 and 2007. Low scores indicate resistance to Colorado potato beetle.

described above. The spatial statistical analyses used in this study differ from those used in previous association studies (Flanders et al. 1992, 1997). Those studies used chi-square analyses, which require subjective classifications of resistance phenotypes in variable populations. The current analyses consider the value of each data point without regard to phenotypic categories.

We searched for significant variables with two strategies. Strategy 1 included all 38 geographic and environmental variables, and strategy two included these 38 and the additional five taxonomic variables (43 in total). For geostatistical calculations, we used the statistical software R and the packages spatstat to plot point patterns (Baddeley and Turner 2005), spdep to test for autocorrelation (Bivand et al. 2006), and spatial for testing the models for any significant contribution of the spatial autocorrelations by using ANOVA (Venables and Ripley 2002). Maps were constructed using the packages maps (Brownrigg et al. 2007) and maptools (Lewin-Koh 2008) in R.

## Results

**Taxonomy.** A severe Colorado potato beetle infestation was observed in both years, although beetles moved into the field later in the summer in 2006 than in 2007. Defoliation levels in the susceptible Red Norland were 85–95% on the second to last score date in both years. Most Red Norland plants were completely defoliated by the last score date. In contrast, no defoliation was observed in the single accession of *S. immite* and four accessions of *S. pinnatisectum* in the trial. In addition, no defoliation was observed in the four accessions of *S. chacoense* in 2006.

Statistical analyses produced similar results in both years. Unless stated otherwise, comparisons refer to both years. There was a significant effect of series (2006:  $F = 7.73$ ,  $df = 13$ ,  $P < 0.0001$ ; 2007:  $F = 36.73$ ,

$df = 13$ ,  $P < 0.0001$ ) (Fig. 2), but not replication or the replication  $\times$  series interaction. The most resistant series were *Yungasensa* and *Commersoniana*, whereas the most susceptible was *Conicibaccata*. There was also a significant effect of clade (2006:  $F = 5.71$ ,  $df = 4$ ,  $P < 0.0002$ ; 2007:  $F = 42.41$ ,  $df = 4$ ,  $P < 0.0001$ ) (Fig. 3) but not the replication  $\times$  clade interaction. Clade one was significantly more resistant than clade four in both years. In addition, in 2006, clade one was significantly more resistant than clade 3. No other significant differences between clades were detected.

There was a significant effect of ploidy (2006:  $F = 25.66$ ,  $df = 2$ ,  $P < 0.0001$ ; 2007:  $F = 144.58$ ,  $df = 2$ ,  $P < 0.0001$ ) (Fig. 4) but not the replication  $\times$  ploidy interaction. All three ploidy levels were significantly different from each other. Diploid plants were the most resistant, followed by hexaploids and then tetraploids. There was a significant effect of EBN (2006:  $F = 14.35$ ,  $df = 2$ ,  $P < 0.0001$ ; 2007:  $F = 110.50$ ,  $df = 2$ ,  $P < 0.0001$ ) (Fig. 5) but not the replication  $\times$  EBN interaction. The two EBN and four EBN species were not significantly different from each other, but they were more susceptible than the one EBN species.

There was a significant effect of species in both years (2006:  $F = 12.02$ ,  $df = 40$ ,  $P < 0.0001$ ; 2007:  $F = 66.35$ ,  $df = 40$ ,  $P < 0.0001$ ) (Fig. 6), and a significant species by replication interaction in 2007 ( $F = 7.06$ ,  $df = 39$ ,  $P < 0.0001$ ) but not in 2006. The most resistant species were *S. immite* Dunal and *S. pinnatisectum*, whereas the most susceptible was *S. tuquerrense* in 2006 and *S. bukasovii* in 2007. When species were ranked by AUDPC, the nine most resistant species were identical in both years (*S. chacoense*, *S. immite*, *S. pinnatisectum*, *S. albornozi* Correll, *S. polyadenium*, *S. neorossii* Hawkes and Hjert, *S. tarijense*, *S. jamesii*, and *S. commersonii* Dunal).

**Spatial and Environmental Variables.** Fig. 1B–D shows interpolated data by using “ordinary kriging” of the square root of AUDPC (SAUDPC). B is based on

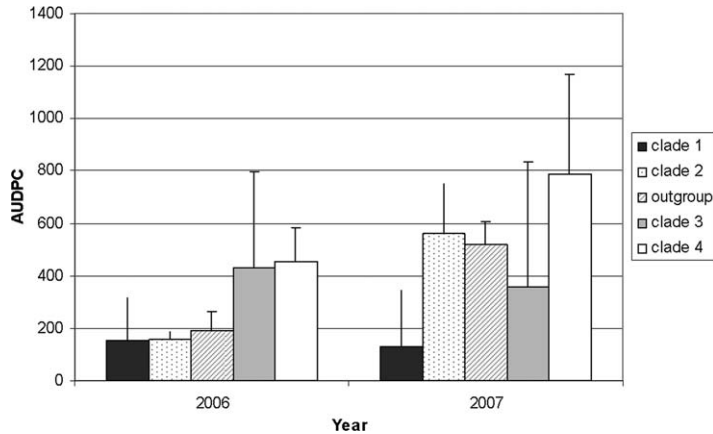


Fig. 3. Mean AUDPC scores for the four clades in sect. *Petota* and one outgroup in 2006 and 2007. Low scores indicate resistance to Colorado potato beetle.

a standard simple interpolation algorithm (kriging) of the observed values of the SAUDPC without using any special statistical model to serve as a reference for Fig. 1C and D; Fig. 1C is based on predicted SAUDPC by using a general linear model, which uses six out of 38 environmental variables that were determined to significantly contribute to the model; Fig. 1D is based on predicted SAUDPC by using a general linear model, which uses one out of 43 variables: species. The interpolated Colorado potato beetle resistance data (Fig. 1B) show a weak trend for resistances in the far north and far south of the distribution of wild potatoes, and in northern Peru. However, overall geographic spatial autocorrelations were only weakly significant ( $P = 0.051$ ) when they were included in the GLM that included the significant environmental and taxonomic variables. Of the 38 geographic variables examined in strategy 1, six were significant: mean monthly minimum temperatures in March and November (temperature data months for southern hemisphere, reversed for northern hemisphere; see *Materials and Methods*), mean monthly maximum temperatures in April and

June, and mean monthly mean precipitation in May and September. As shown in Fig. 1C, there is no clear trend in predictivity of Colorado potato beetle resistance to geographic areas or any clear relationship to the observed resistance data (Fig. 1B). In strategy two using all 43 variables (including the taxonomic ones), only the variable species remained in the model. This single variable, as expected, has some relationship in the GLM model (Fig. 1D) to the observed values (Fig. 1B) in showing a weak trend of Colorado potato beetle resistance in the far north and far south of the distribution of wild potatoes, and in northern Peru.

## Discussion

During the field season in temperate regions, adult Colorado potato beetles emerge from overwintering sites adjacent to cultivated fields in late spring. They colonize emerging potato plants in early summer and lay eggs. Larvae emerge from the eggs in midsummer and pass through four instar stages over a period of about three weeks. Fourth instar larvae then drop into

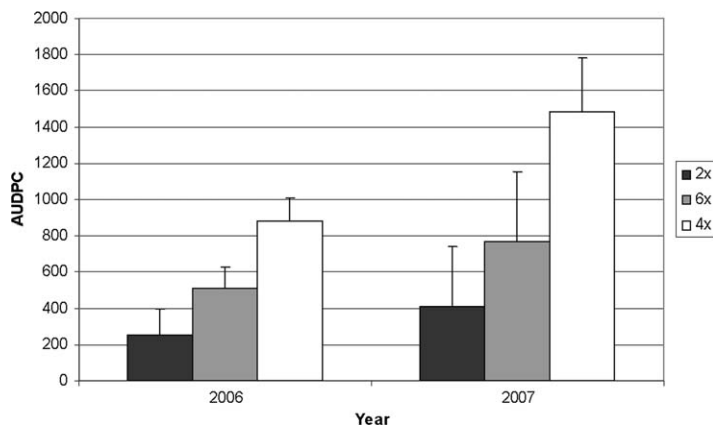


Fig. 4. Mean AUDPC scores for the three ploidy levels in sect. *Petota* in 2006 and 2007. Low scores indicate resistance to Colorado potato beetle.

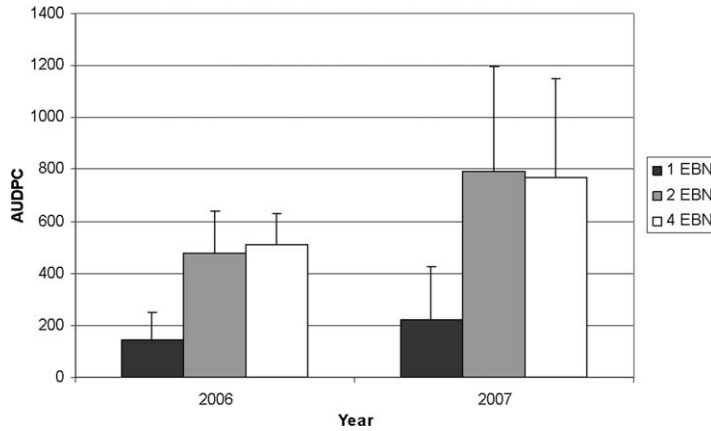


Fig. 5. Mean AUDPC scores for the three endosperm balance numbers in sect. *Petota* in 2006 and 2007. Low scores indicate resistance to Colorado potato beetle.

the soil, where they pupate and emerge as adults  $\approx 2$  wk later. Most defoliation damage results from late-instar larvae. We designed our experiment as a free choice feeding assay under commercial field conditions. Defoliation estimates were measured regularly throughout the period when most (>60%) beetles in the field were large larvae (e.g., fourth instars). In

2006, adult beetles moved into the Colorado potato beetle screening field in late July, so defoliation damage by larvae was first detected in early August. Consequently, scoring of defoliation was carried out at weekly intervals on four dates in August. By late August, most Colorado potato beetles in the field were adults, so scoring ceased. In 2007, adult beetles moved

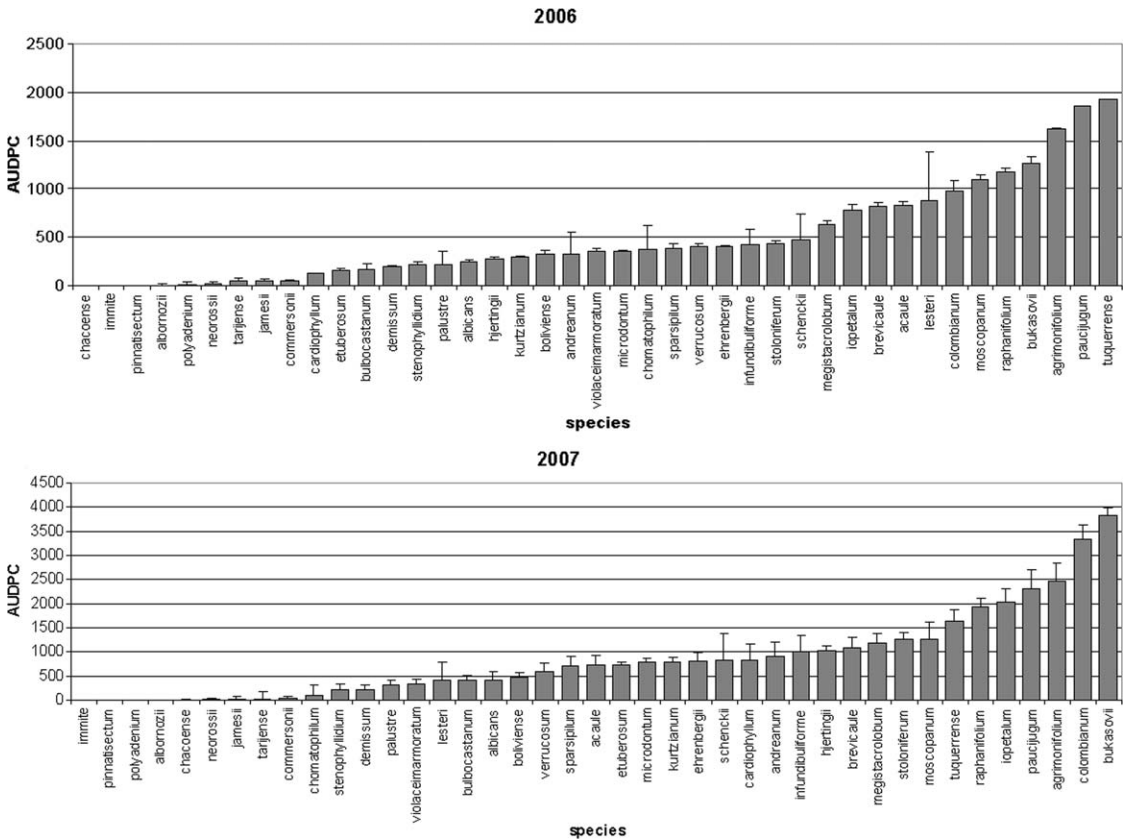


Fig. 6. Mean AUDPC scores for 41 species in 2006 and 2007. Low scores indicate resistance to Colorado potato beetle.

into the field earlier, in late June. Scoring was therefore carried out at weekly intervals throughout July and August, when most of the beetles were larvae. Two generations of beetles were produced during this interval. Although this study did not distinguish between antixenosis and antibiosis, it was meant to provide a general measure of resistance to larval feeding on a broad array of species and accessions.

In a field study using insects migrating from hedgerows into research plots, a uniform distribution of feeding pressure is of concern. Our experimental field was blocked so that one replication was close to the hedgerow and the second was more distant, but was adjacent to other potato fields. In both years, we consistently observed no effect of block on defoliation scores. In addition, 'Red Norland' check plants were uniformly defoliated. Consequently, it seems that colonization of the field was uniform.

The nine most resistant species in both years were *S. chacoense*, *S. immite*, *S. pinnatisectum*, *S. albornozi*, *S. polyadenium*, *S. neorossii*, *S. tarijense*, *S. jamesii*, and *S. commersonii*. These results were similar to those of (Flanders et al. 1992), who identified *S. chacoense*, *S. pinnatisectum*, *S. polyadenium*, *S. tarijense*, and *S. jamesii* as the most resistant species in a broad survey of Colorado potato beetle resistance in 100 *Solanum* species. An additional three species (*S. immite*, *S. neorossii*, and *S. commersonii*) were resistant in our study, but susceptible in the Flanders et al. (1992) study. Perhaps variation among wild species accessions or Colorado potato beetle populations account for these differences.

Unlike our previous studies of resistance to foliar fungi (Jansky et al. 2006, 2008), we found some consistency among accessions within a species and plants within an accession, especially with the most resistant species. In both years, there was no variability among accessions for the three most resistant species in which multiple accessions were evaluated (*S. pinnatisectum*, *S. polyadenium*, and *S. albornozi*). In fact, all plants of *S. immite* and *S. pinnatisectum* were uniform in that they did not exhibit any defoliation throughout both growing seasons. Flanders et al. (1992) also reported that all 10 accessions of *S. pinnatisectum* tested were resistant, whereas only one accession of *S. immite* was tested. Considering the nine most resistant species in this study, only one accession was available for evaluation of *S. immite* and *S. neorossii*, so interaccession variability could not be measured. Of the remaining seven species in each of two years, there was no variation among accessions in 11 of those 14 comparisons. In contrast, interaccession variability was reported in the Flanders et al. (1992) study.

The effect of glycoalkaloids on foliar feeding by Colorado potato beetle is well documented (Sinden et al. 1986, Lawson et al. 1993, Rangarajan and Miller 2000). In particular, leptine glycoalkaloids are especially attractive as a source of Colorado potato beetle resistance because they are very effective larval feeding deterrents and their production is limited to foliage. Consequently, human health concerns associated with glycoalkaloid consumption are absent when in-

corporating leptines into potato cultivars. To date, leptine glycoalkaloids have only been found in *S. chacoense*. In our study, *S. chacoense* was among the most resistant species in both years, probably due to high glycoalkaloid levels. In 2007, there was no defoliation in three *S. chacoense* accessions and some defoliation in the fourth accession (320285), making it significantly different from the others. In 2006, there was no defoliation in any of the four accessions. In contrast, a large amount of variation in Colorado potato beetle resistance has been reported among and within *S. chacoense* accessions (Torka 1950, Sinden et al. 1986). In the Torka (1950) study, total foliar glycoalkaloid levels were similar, but resistance levels varied due to differences in leptine glycoalkaloid levels. We are currently evaluating types and levels of glycoalkaloids in this and other resistant species identified in this study.

Two of the nine most resistant species were *S. polyadenium* and *S. tarijense*. Both of these species have been reported to be resistant to Colorado potato beetle due to high levels of glandular trichomes (Gibson 1976, Dimock and Tingey 1988). We noted dense levels of glandular trichomes on the leaves of both species. In both years, there was no significant variation in resistance scores among accessions of either species. Consequently, it seems that these species are not preferred by the Colorado potato beetle. However, although these two species possess similar insect resistance mechanisms, they are not closely related to each other. *S. polyadenium* is a member of clade 1, whereas *S. tarijense* is in clade 4. Consequently, species that produce glandular trichomes are not clustered within a particular taxonomic group.

Pelletier and Dutheil (2006) have reported that Colorado potato beetle resistance mechanisms seem to differ between *S. berthaultii* and *S. tarijense*. In *S. tarijense*, glandular trichomes are dense, but do not have a strong phagodeterrent effect. Foliage consumption by adults is similar when trichomes are removed. In contrast, *S. berthaultii* trichomes act as an important feeding deterrent. However, only one accession of each species was considered in the study. In addition, Flanders et al. (1992) suggest that these species exhibit differences in their responses to several insect pests. However, a taxonomic evaluation of many accessions (Spooner and van den Berg, 1992) has resulted in the grouping of the two species into one species, *S. berthaultii* (Spooner et al. 2007).

Two of the nine highly resistant species (*S. pinnatisectum* and *S. jamesii*) have been reported to be resistant to Colorado potato beetle in previous studies, although their resistance mechanisms have yet to be elucidated (Flanders et al. 1992, Pelletier and Tai 2001, Li et al. 2006). Three of the most resistant species (*S. pinnatisectum*, *S. polyadenium*, and *S. jamesii*) are found in Mexico and the southwest United States, so they grow within the geographic range of the Colorado potato beetle. However, the Colorado potato beetle is found at lower elevations than that of the wild *Solanum* species, so they may

not occupy the same environments (Flanders et al. 1992). Six of the nine most resistant species are not even found within the geographic range of the Colorado potato beetle. It is possible that the traits responsible for Colorado potato beetle resistance in this study confer some other type of survival advantage in other regions. For example, glandular hairs protect wild potato plants against spider mites (Gibson 1979) and aphids (Gibson 1971). Alternatively, resistance may be incidental, due to secondary metabolites or leaf characteristics (Flanders et al. 1992).

The best sources of Colorado potato beetle resistance seem to be the wild diploid *Solanum* species. Within that group, the one EBN species are the most resistant. These species are commonly reported to be rich sources of resistances to diseases, pests, and environmental stresses (Hawkes 1990a; Valkonen et al. 1992; Cardi et al. 1993; Helgeson et al. 1998; Carputo et al. 2000; Kuhl et al. 2001; McGrath et al. 2002; Zlesak and Thill 2004; Jansky et al. 2006, 2008). Flanders et al. (1992) suggested that insect resistance in *Solanum* is a primitive trait. Hawkes (1990) has proposed that the most primitive tuber-bearing species were one EBN, which supports our observation that one EBN species are rich sources of Colorado potato beetle resistance. Unfortunately, these species are also more difficult to introgress into the cultivated potato than are diploid two EBN species. Chromosomal rearrangements have been reported in the diploid one EBN species *S. pinnatisectum*, one of the most resistant species in this study (Li et al. 2006). This may limit opportunities for introgression of Colorado potato beetle resistance genes into cultivated germplasm.

The most resistant series were *Yungasensa* and *Commersoniana*, due to high levels of resistance in *S. chacoense* and *S. tarijense* (*Yungasensa*) and *S. commersonii* (*Commersoniana*). In contrast, Flanders et al. (1992) reported that Colorado potato beetle resistance was most common in the superseries *Stellata* (which includes series *Bulbocastana* and *Pinnatisecta*) and series *Piurana*. In our study, series *Bulbocastana*, *Pinnatisecta*, and *Piurana* ranked 4, 3, and 7, respectively, out of 14 series in both years. The two studies used different sets of species to represent each series, so it may not be surprising that rankings were not the same. Based on both studies, it seems that series *Bulbocastana* and *Pinnatisecta* are rich sources of Colorado potato beetle-resistant species.

There was no significant association of Colorado potato beetle resistance in wild potato species to spatial data. Altitude data were available for six of the nine most resistant species in our study. It is interesting to note that all accessions in five of those species were collected exclusively at high elevations (>1,500 m). Only *S. commersonii* was collected at low elevations (50 m). In contrast, Flanders et al. (1997) found that accessions from low elevations were significantly more resistant to the Colorado potato beetle than were those from high elevations. Perhaps elevation is not a critical element in the distribution of Colorado

potato beetle resistance. For example, all accessions of *S. jamesii* and *S. tarijense* (except one) were collected from low elevations in the Flanders et al. (1997) study, but all accessions used in our study came from high elevations. Regardless of elevation, these species were resistant in both studies.

We detected a weak association between Colorado potato beetle resistance and distribution in the far north and far south of the range of *Solanum* species and in northern Peru. Flanders et al. (1997) found more Colorado potato beetle resistance than expected in species from southwestern Mexico, Bolivia, and Argentina. Resistance was detected in species from all of these regions, but there were many susceptible species from these regions as well. Resistance is not concentrated enough to provide guidance regarding geographic localities likely to contain a high proportion of populations containing Colorado potato beetle resistance.

It is difficult to find a simple explanation for the six environmental variables that were shown to be significantly associated in the GLM model. At best, this could be interpreted as some environmental niche, but these six variables show no evident associations to us. When included in a GLM analysis using species data, they lose all significance. Hence, the environmental variables may be artifactual or at best not readily interpretable in an ecological sense.

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