

# Effects of the Pesticide *Furadan* on Traits Associated with Reproduction in Wild Potato Species

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## ABSTRACT

Natural populations of wild potato species are the backups for the diversity held in genebanks for research and breeding. Some potato species are known to grow in close proximity to cultivated fields, thus are potentially impacted by human activity, including exposure to pesticides. The present study tested the effects of a common pesticide on reproductive traits of potatoes known to grow in or near pesticide-treated fields in central Peru. Furadan<sup>®</sup> 4F, an insecticide—nematicide (common name = *carbofuran*) was applied at two different rates to populations representing 15 wild potato species in a greenhouse environment in Peru. Flowering duration of these populations was usually significantly reduced in comparison to a water control, and in a few cases, percent viable pollen also was. These findings suggest that agrichemicals may be having unintentional effects on wild potato populations in ways that could compromise their genetic diversity.

**Keywords:** Potato Species; Pesticide Contamination; Genetic Drift; Reproductive Capacity

## 1. Introduction

Wild potato populations are being impacted by human activity, most extremely when they go extinct, *per se*, as a result of having their habitats converted to some other uses (Coca-Morante and Castillo-Plata, 2007) [1]. But more subtle influences may be occurring, perhaps only eliminating specific traits or otherwise narrowing the genetic base of populations.

According to a recent taxonomic estimate by Spooner (2009) [2], potato wild relatives are represented by about 100 species which are distributed in many different types of eco-geographic niches from the southwestern USA to southern Chile (Hijmans and Spooner, 2001) [3]. Some are known to grow within or very close to fields in which potato or other crops are cultivated. Pesticides are used in virtually all potato fields in the Andean region. These fields receive up to seven pesticide applications per season with each application averaging 2 - 3 different products (Yanggen *et al.*, 2003) [4]. Carbofuran and metamidofos are the major insecticides used and constitute 47% and 43%, respectively, of all the active ingredients applied (Forbes *et al.*, 2009) [5].

Flowering and pollen production are essential in plant sexual reproduction, so changes could impact genetic diversity and population fitness (Ennos 1994 [6]; Hamrick *et al.*, 1979 [7]; Loveless and Hamrick, 1984 [8]).

There is evidence that agrichemicals can alter plant biochemistry and metabolic mechanisms as well as disrupt mitotic and meiotic cycles in plants. Application of pesticides was related to the modulation of the biosynthetic pathways leading to polyphenol formation in peas, oats, potatoes, raspberries, etc. (Daniel *et al.*, 1999) [9] and Lydon and Duke (1989) [10] reported significant changes in the content of secondary compounds. Asita and Matebesi (2010) [11] found that pesticides caused cytotoxic and genotoxic effects in root meristems of *Allium cepa*, *Vicia faba* and *Zea mays*. These effects varied from point mutations to chromosome aberrations. Other studies indicated that fungicides affected germination, mitotic and meiotic activity, and pollen fertility in barley and tomatoes (Behera *et al.* 1982 [12]; Fairbanks *et al.* 2002 [13]; Tort *et al.* 2005 [14]), pollen viability in tomatoes (Cali and Candan 2009) [15] and flower production in different species (Spiers *et al.* 2006) [16]. The literature, however, does not report impacts of agrichemicals in species related to potatoes.

Ahmad *et al.* (1979) [17] reported that site-specific environmental variables determine persistence of pesticides and their biological impact. For example, while carbofuran degrades rapidly at high soil pH, Finlayson *et al.* (1979) [18] estimated it has a half-life of about 16 years in soils of pH 5.5. The pH of the Andes soils, from which the species tested in our study originated, are

known to be very acidic—sometimes lower than pH 4.0 (Arica *et al.* 2006) [19]. It follows that yearly applications could result in buildup of carbofuran in the soil. For this reason, the effects of exposure of wild species to levels higher than the recommended rate were also of practical interest.

The present study was initiated to assess flowering and pollen viability effects in wild potato populations after controlled application of carbofuran, a pesticide to which they would likely be unintentionally exposed in the farm field setting.

## 2. Methods and Materials

### 2.1. Plant Material

Twenty-one populations from 15 different potato species from diverse regions in the Andes were selected because

of their known natural proximity to farm fields. Plants used in this study originated as botanical seed from CIP's potato germplasm collection. **Table 1** provides identities and other details of the plant material used.

### 2.2. Pesticide Selection and Application

A survey of farmer's communities at Quilca, Colpar and Nunhuayo in the Mantaro Valley confirmed that Furadan<sup>®</sup> as the most consistently-used pesticide in the region (100% of farmers). The responses were gathered from interviews made to 14 potato farmers who were leaders and responsible of agricultural decisions in their respective communities. Furadan<sup>®</sup> is the commercial name for Carbofuran (*2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate*), a carbamate with insecticidal and nematocidal properties resulting from inhibition

**Table 1. Identities and origins of wild potato populations used in this study.**

<i>Solanum</i> ...	Population code <sup>b</sup>	Chromosome number	Original location (site, province, elevation) <sup>c</sup>
<i>acl</i>	OCH 11322	48	Huaro-chiri, Lima, 3154 m
<i>acl</i>	OCHS 11889	48	Acora, Puno, 3840 m
<i>alb</i>	OCH 11842	72	Kawish, Ancash, 4200 m
<i>alb</i>	OCHS 16023	72	Shojlla, Cajamarca, 3550 m
<i>amb</i>	OCHS 11865	24	Choccec, Pasco, 2800 m
<i>buk</i>	HJT 5444	24	Lircay, Huancavelica, 3800 m
<i>buk</i>	OCH 13713	24	Ckumuccacca, Cusco, 3650 m
<i>chq</i>	OCH 13963	24	Cajon, Cajamarca, 2850 m
<i>hcr</i>	OCH 11692	24	Llusuppuquio, Ancash, 2800 m
<i>lgl</i>	OCH 11617	24	Intihuatana, Cusco, 2800 m
<i>lmb</i>	SS 7205	24	Phara, Puno, 3640 m
<i>med</i>	OCH 12044	24	Surco, Lima, 2240 m
<i>med</i>	SSTS 7302	24	Hongos, Lima, 2880 m
<i>mga</i>	OCH 11941a	24	Mollepunco, Potosi <sup>d</sup> , 3880 m
<i>mtp</i>	OCHS 11307	24	Canta, Lima, 2800 m
<i>rap</i>	HHCH 5141	24	Sacsayhuaman, Cusco, 3550 m
<i>rap</i>	OCH 13626	24	Paucarpata, Cusco, 3800 m
<i>spl</i>	SS 7213	24	Tarapata, Cusco, 2900 m
<i>trp</i>	OCHB 15687a	24	Luruchayoc, Cusco, 3400 m
<i>trp</i>	SS 7216	24	Tarapata, Cusco, 3200 m
<i>uru</i>	SS 7225	24	Vilcabamba, Cusco, 2600 m

<sup>a</sup>Potato species abbreviations: *Solanum acaule* (*acl*), *S. albicans* (*alb*), *S. ambosinum* (*amb*), *S. bukasovii* (*buk*), *S. chiquirense* (*chq*), *S. hypacanthum* (*hcr*), *S. lignicaule* (*lgl*), *S. limbanense* (*lmb*), *S. medians* (*med*), *S. megistacolobum* (*mga*), *S. multiinterruptum* (*mtp*), *S. raphanifolium* (*rap*), *S. sparsifolium* (*spl*), *S. tarapatanum* (*trp*) and, *S. urubambae* (*uru*); <sup>b</sup>Population code represents the original collector's identifier as listed in CIP's germplasm database; <sup>c</sup>All but one of the provinces listed in the table are located in Peru; m = meters above sea level; <sup>d</sup>Province of Bolivia.

of cholinesterase, which catalyzes the neurotransmitter acetylcholine. Furadan<sup>®</sup> is systemic, with high water solubility and rapid uptake from roots and leaves (PAN 2008) [20].

The experiment was conducted in the greenhouses at CIP's Santa Ana Station in Huancayo, Peru (12.067°S × 75.217°W; 3380 m) in the summer (January-March). Each of the 21 populations was replicated 3 times in a Completely Random Design with experimental units composed of 5 seedlings each. Seeds were sown and transplanted into 20 cm plastic pots filled with a mix of soil and peat moss, and fertilized and hand watered as standard for optimal growth. Furadan<sup>®</sup> in liquid form (480 g/L active ingredient) was obtained from Farmagro S.A., Lima. Two treatment concentrations (per liter) were applied:  $trt_{3.5} = 3.5$  ml,  $trt_{4.5} = 4.5$  ml, and compared to control plants. Leaves of all plants were sprayed to drip a total of 5 times at 10-day intervals over the growing season. The control plants (similarly sprayed but with pure water) were grown in a separate greenhouse to prevent accidental exposure to the pesticide.

### 2.3. Trait Evaluation

Flowering was considered to have begun when at least one plant in the experimental unit had five inflorescences in bud stage, and to have ended when all flowers had dried up. Plants were inspected daily to see if these conditions were met. Pollen was collected from each population at estimated peak flowering, from at least one flower from each plant in the experimental unit. Pollen grains were collected by mechanical vibration using a doorbell buzzer and were examined microscopically in a 2% solution of acetocarmine glycerol. The average percent stained pollen was recorded for three random fields of view at 40×. Acetocarmine is not a vital stain per se, but staining indicates that normal pollen development has occurred, so unstained grains were considered inviable.

Analysis of variance was conducted using JMP statistical software (JMP9 2011) [21] to calculate treatment's LSD in each trait assessed. In the test of pollen viability, percent data were arcsine transformed before analysis.

## 3. Results and Discussion

### 3.1. Flowering Duration

Populations varied in control flowering duration from 30 to 79 days (see **Table 2**). The standard application of Furadan ( $trt_{3.5}$ ) commonly depressed flowering duration at average of 25% relative to controls (from 48 to 36 days), which was significant ( $p < 0.05$ ) for 17 of the 21 populations examined. Populations with more robust control flowering tended to be reduced more in response to Furadan exposure. Note for example, that the four

populations which did not show significant reduction in response to Furadan application all had among the lowest control flowering. The mechanism responsible for the observed differences in pesticide sensitivity is not known, but it is common for a pesticide to be toxic to one plant species and not to another (Dalvi *et al.* 1972 [22]; Pereira *et al.* 2010 [23]). Increasing the dosage to  $trt_{4.5}$  did not often result a significant additional reduction of flowering duration—being the case in only for three of the populations.

### 3.2. Stainable Pollen Percent

Pollen viability estimates were very high in control, averaging 91% and ranging from 75% to 100% (see **Table 2**). Standard Furadan application ( $trt_{3.5}$ ) reduced percent stainable pollen by an average of only about 7%, and only significantly in 3 of the 21 populations. The greatest effect was found in populations of buk and lgl, where pollen viability was reduced by 25% and 24%, respectively. In three other populations, the higher dose ( $trt_{4.5}$ ) resulted in significant reduction compared to control. While Furadan treatment never resulted in a substantial increase in flowering duration, in one case (med-OCH 12044), it appeared to *increase* percent stainable pollen. This coincided with particularly low percent stainable pollen in the corresponding control plants. This observation implies need for follow-up experiments to confirm the pollen viability-enhancing effect of Furadan and identify the sub-optimal control environment conditions that the presence of Furadan apparently improves in some germplasm.

This experiment was not designed to differentiate species according to Furadan effects, nor associate flowering and pollen effects in a given population. However, results in **Table 2** do not suggest any obvious pattern of species susceptibility, and the correlation between Furadan impact on flowering and pollen viability is a non-significant  $-26\%$ , suggesting no obvious link between the two.

### 3.3. Potential Impact on Potato Genetic Diversity

*Solanum* species and other native plants in the Andes often compete for the same few insect pollinators that are available at high elevations. Altered flowering could divert bees to other plants and impact potato reproduction potential by suppressing (or limiting) pollination (Husband and Schemske 1996) [24]. The prospects of population genetic impact seem particularly relevant considering that majority of the potato species examined here are diploid obligate out crossers (**Table 1**)—a breeding system that is vulnerable to genetic drift when effective population size is restricted (Bamberg and del Rio 2004 [25]; Loveless and Hamrick 1984) [8].

**Table 2. Effects of Furadan treatments on flowering and pollen of wild potato species.**

Species <sup>1</sup>	Population	Flowering Duration (Days) <sup>2</sup>			Stainable Pollen Percent <sup>3</sup>		
		Control	trt <sub>3,5</sub>	trt <sub>4,5</sub>	Control	trt <sub>3,5</sub>	trt <sub>4,5</sub>
<i>acl</i>	OCH 11322	53	37*	36	99	95	93
<i>acl</i>	OCHS 11889	52	24*	21	100	99	87
<i>alb</i>	OCH 11842	79	53*	48	99	95	93
<i>alb</i>	OCHS 16023	69	57*	51	97	93	92
<i>amb</i>	OCHS 11865	39	27*	22	75	83	67*
<i>buk</i>	HJT 5444	36	36	31	95	71*	89
<i>buk</i>	OCH 13713	51	44*	41	94	88	90
<i>chq</i>	OCH 13963	49	38*	36	90	83	81
<i>hcr</i>	OCH 11692	50	34*	24*	84	76	77
<i>lgl</i>	OCH 11617	49	39*	33	96	73*	70
<i>lmb</i>	SS 7205	62	42*	41	91	84	79
<i>med</i>	OCH 12044	42	37	28*	78	91+	90
<i>med</i>	SSTS 7302	43	36*	31	93	89	89
<i>mga</i>	OCH 11941a	38	23*	23	82	73	73
<i>mtp</i>	OCHS 11307	40	26*	27	91	80	88
<i>rap</i>	HJTCH 5141	30	31	23*	93	81*	90
<i>rap</i>	OCH 13626	50	37*	34	96	96	97
<i>spl</i>	SS 7213	39	24*	22	93	94	94
<i>trp</i>	OCHB 15687a	35	29	30	92	84	78*c
<i>trp</i>	SS 7216	57	36*	40	78	75	68*c
<i>uru</i>	SS 7225	52	39*	37	96	87	84*c
<b>Averages</b>		<b>48</b>	<b>36*</b>	<b>32</b>	<b>91</b>	<b>85*</b>	<b>84</b>

<sup>1</sup>See **Table 1** for full species names; <sup>2</sup>LSD<sub>0.05</sub> flowering duration = 8.53; <sup>3</sup>LSD<sub>0.05</sub> stainable pollen percentage pollen = 10.63; Control = water, trt<sub>3,5</sub> = 3.5 ml/L, trt<sub>4,5</sub> = 4.5 ml/L; + = significantly greater than control; trt1\* = significantly less than control, trt2\* = significantly less than trt1 and control; trt2\*c = significantly less than control but not sign less than trt1.

The significance of human influence on *in situ* germplasm depends on the sensitivity of the germplasm in question, the level of exposure, and the value/rarity of the germplasm with respect to agriculture and the ecosystem. This preliminary survey demonstrated that the pesticide Furadan, to which *in situ* germplasm may be expected to have high exposure, can indeed have a negative influence on reproductive indicators in greenhouse-grown plants. More focused follow-up studies are in progress to better characterize the patterns and mechanisms by which these reproductive traits are depressed, and quantify the consequences on genetic diversity that could logically follow in the wild.

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