

## ELEVATED HERBIVORY IN PLANT HYBRID ZONES: *CHRYSOMELA CONFLUENS*, *POPULUS* AND PHENOLOGICAL SINKS<sup>1</sup>

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**Abstract.** Using the free-feeding beetle *Chrysomela confluens* (Coleoptera: Chrysomelidae), we examined the hypothesis that naturally occurring hybrid zones between two cottonwood species are “sinks” or centers of insect abundance. Over a 3-yr period, we found that 94% of the *C. confluens* population was restricted to a 13-km hybrid zone, which represents <3% of the cottonwood population.

Of several potential mechanisms tested, expanded host phenology in the hybrid zone, relative to pure zones, best explained the distribution of *C. confluens* which is dependent upon newly flushed leaves for optimal growth and reproduction. The hybrid zone is a superior beetle habitat because: (1) early leaf flush in the hybrid zone provides the first source of food for beetles in spring and (2) staggered leaf phenologies in the hybrid zone allow beetles to shift onto newly flushed Fremont trees as foliage of sympatric hybrid and narrowleaf trees declines in quality. This shift by ovipositing females can result in a 600% increase in fecundity relative to nonshifting females. Additionally, a 10-yr common garden experiment shows that there is a strong genetic component to the timing of leaf flush, which suggests a long-term stability of resources in the hybrid zone that beetles can rely on.

For *C. confluens*, the hybrid zone is a “phenological sink” that increases beetle fecundity and leads to chronically high herbivory year after year. Because movement from the hybrid zone reduces fecundity, emigration into adjacent pure zones is likely hindered. This hypothesis requires staggered phenologies between overlapping host species; it probably does not require hybridization between host species. Further, this hypothesis may in part explain frequent outbreaks of insects in nurseries and plantations. Thus, two distinct mechanisms have been identified that result in increased insect abundance in plant hybrid zones: “phenological sinks” resulting from staggered plant phenologies and “hybrid sinks” resulting from increased susceptibility of hybrids to insect attack. Such studies suggest that hybrid zones are important natural laboratories for the study of plant–herbivore interactions.

**Key words:** chronic herbivory; Chrysomelidae; cottonwood; host shifting; hybrid sink; hybridization; phenological sink; poplar.

### INTRODUCTION

Plant hybrids have been neglected in studies of insect–plant interactions despite the fact that hybridization is common to all major plant taxonomic groups (Grant 1971) and may account for the origins of 30–80% of all plant species (Stace 1987, Wendel et al. 1991). In part, this is because the systematics of many plant taxa are unclear given the propensity of these groups to hybridize (e.g., syngameons and “ring” species; Grant 1971). Further, “pure” plants may not be easily distinguished from complex backcrosses or backcrosses from F1 hybrids. Finally, the relative rarity of hybridization between animal species may encourage nonbotanists in the belief that hybridization between plant species is an equally anomalous event and of no intrinsic value (see O’Brien and Mayr 1991 and re-

sponse by Whitham et al. 1991). For these reasons, plant hybridization is probably viewed more as a complicating factor to be avoided than one to be studied.

Plant hybrid zones, however, can act as “sinks” or centers of insect abundance, which may have ecological and evolutionary implications for herbivorous insects. For example, 85–100% of the *Pemphigus betae* aphid population in Weber Canyon, Utah, is restricted to *Populus fremontii* × *P. angustifolia* backcross hybrids that represent <3% of the host population (Whitham 1989). Aphids colonizing these hybrids have higher fitness than aphids on the ancestral host species, *P. angustifolia*, possibly due to a hybrid breakdown of the tree’s defenses (Keim et al. 1989, Whitham 1989, Paige et al. 1990). Based on these findings, Whitham (1989) proposed that plant hybrid zones might act as “sinks” whereby relaxed selection pressures associated with susceptible hybrid hosts could prevent the progeny of colonizing herbivores from adapting to the pure host

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population. If so, susceptible hybrids represent "sinks" in evolutionary time with important implications for both pest management (Atsatt and O'Dowd 1976, Gould 1986, Whitham 1989) and bioconservation (Whitham et al. 1991, Floate 1992). For example, elimination of plant hybrid zones could result in the loss of pest and nonpest species restricted to them (Whitham et al. 1991).

If we are to assess the importance of hybrid zones in affecting the ecology and evolutionary biology of insects, it is necessary to understand both the general patterns of insect distribution and their underlying mechanisms. For example, one study that contradicts Whitham's (1989) "hybrid sink" hypothesis is by Boecklen and Spellenberg (1990) in which they found that oak (*Quercus*) hybrids typically supported fewer individuals and species of herbivores than did the parental species. However, because the pattern of plant hybridization (i.e., sterile F1 progeny vs. fertile F1's that backcross with one or more of the parental species) affects conclusions drawn from such studies (Boecklen and Spellenberg 1990, Floate and Whitham 1993), and since the type of hybridization between oak species was unknown, explanations for the differences between *Populus* and *Quercus* systems are unavailable.

In the present study, we examined the "sink" hypothesis along the Weber River in northern Utah using the free-feeding beetle *Chrysomela confluens*. The hypothesis was first developed in this system using the specialist, gall-forming aphid *Pemphigus betae*. Thus, our choice of *C. confluens* allowed us to examine the generality of the hypothesis by comparing insects with very different life cycles. The goals of this study were twofold. First, we wanted to determine whether the distribution of *C. confluens* was centered in the hybrid zone. This being the case, we then examined three potential mechanisms that might explain this distribution. (1) The "phenological sink" hypothesis. Because chrysomelid species preferentially feed on newly flushed leaves (e.g., Raupp and Denno 1983), the extended availability of these leaves in the hybrid zone increases beetle fitness relative to the fitness of beetles in adjacent pure zones. (2) The "hybrid sink" hypothesis. The greater susceptibility of hybrid, relative to pure, hosts increases insect numbers in the hybrid zone (Whitham 1989). (3) The "distributional pulse" hypothesis. The concentration of beetles in the hybrid zone is transitory and shifts up an elevational gradient to reflect local changes in temperatures during the summer.

## METHODS

### *The system*

*Chrysomela confluens* (Coleoptera: Chrysomelidae) feeds and oviposits primarily on reproductively immature Fremont, *Populus fremontii*, and narrowleaf, *P. angustifolia*, cottonwood and their hybrids (Kearsley

and Whitham 1989). In northern Utah, overwintering adults emerge in early April and lay 3–10 clusters of 20–40 eggs each on leaves from late April until late June. Eggs hatch in  $\approx 10$  d and larvae begin feeding on young leaves. Pupation occurs on the host and the new generation of adults appears from late May until early July. New adults drop into the leaf litter in mid-July to begin their overwintering diapause (Kearsley and Whitham 1989). Beetles are reproductively immature until the following spring. Adults are easily manipulated, and because they are aposematic, *C. confluens* can be accurately surveyed.

Chrysomelids exhibit greater preference for, and performance on, young leaves relative to old (Brown 1956, Rockwood 1974, Phillips 1976, Burkot and Benjamin 1979, Harrell et al. 1982, Raupp and Denno 1983, Coleman 1986, Larsson and Ohmart 1988, Ernest 1989, Strauss 1990, Bingaman and Hart 1992). For *C. confluens*, larval developmental time can be reduced 10–17% and mortality by 40–60% when larvae are reared on immature leaves rather than mature leaves of the same shoot (Kearsley and Whitham 1989). Young or immature leaves, defined here as leaves that are still expanding, are more nutritious than mature or fully expanded leaves. Mature leaves are more sclerophyllous with high levels of fiber, lignin and tannin and may lack adequate levels of nitrogen or sugars for insect development (e.g., Coleman 1986 and references within).

In cottonwood, spring budbreak initiates the expansion of leaf primordia formed the previous summer with terminal shoots subsequently producing new leaves into the fall. This phenological pattern occurs throughout the Fremont zone and the lower elevations of the narrowleaf zone. However, a shorter growing season abbreviates or even eliminates summer leaf production at higher elevations (K. D. Floate and T. G. Whitham, *personal observations*). Because *C. confluens* is only active from early April to mid-July, larvae and adults feed mainly on leaves flushed in the spring. How the indeterminate growth of *Populus* affects herbivorous insects has been discussed elsewhere (Niemelä and Haukioja 1982, Niemelä et al. 1982).

Cottonwood grows in riparian habitat along the Weber River. Fremont cottonwood grows at elevations of  $\approx 1300$ – $1500$  m and narrowleaf cottonwood grows at elevations of  $\approx 1400$ – $2300$  m. These species overlap in a 13-km zone where hybridization produces F1 individuals that backcross only to narrowleaf cottonwood (Keim et al. 1989). This unidirectional introgression results in a hybrid zone where trees may be pure Fremont, pure narrowleaf, F1 intermediates, or narrowleaf backcrosses. The boundaries of this zone are those used by Whitham (1989), which were based on the presence of narrowleaf, Fremont, and intermediate types. DNA analyses by Keim et al. (1989) demonstrated that within these boundaries, Fremont cottonwood and complex narrowleaf backcrosses are com-

mon whereas narrowleaf cottonwood and F1 hybrids are relatively rare. More recent analyses (Paige and Capman 1993) show that the boundaries of the hybrid zone have been underestimated and that complex backcrosses closely resembling narrowleaf cottonwood occur in what had previously been described as the pure narrowleaf zone. However, because the conclusions of the present paper are not dependent upon a more precise definition of the limits of hybridization, we use the boundaries originally identified.

#### *Observational determination of beetle distribution*

To determine the distribution of *C. confluens* over the range of its cottonwood hosts, 14 sites were surveyed in Fremont (3 sites), hybrid (4 sites) and narrowleaf (7 sites) zones. Sites were standardized for tree age, distance from the river and openness of the canopy. For example, trees censused for beetles and leaf phenology were reproductively immature and 1–3 m in height. Because cottonwoods often reproduce asexually by “suckering,” trees of the same morphology < 10 m apart were not sampled in an effort to maximize the genetic independence of trees surveyed. Ten trees were censused for adult beetles (2 min/tree) at each site. Single tree counts were then averaged to obtain a site mean and standard error. Censuses were repeated for 3 yr.

Initial surveys indicated that the beetle population was centered in the hybrid zone. The three mechanisms subsequently examined as explanations for this distribution were: the phenological sink hypothesis, the hybrid sink hypothesis, and the distributional pulse hypothesis. Unless otherwise stated, observational and experimental data were analyzed using analyses of variance tests for comparisons between groups using an  $\alpha = .05$ . Mean values are given with  $\pm 1$  SE.

#### *Experimental test of the phenological sink hypothesis*

Greater resource availability due to expanded host phenology in the hybrid zone may account for the distribution of beetles. Chrysomelids attain greatest fitness on immature foliage. If the phenologies of Fremont, narrowleaf, and/or hybrid hosts are staggered, the hybrid zone provides beetles with the opportunity to shift between these hosts and maximize adult fitness. Two studies were initiated to test this hypothesis.

First, to document when food for *C. confluens* first becomes available in the spring, 10 immature trees were censused at each of 14 pure and hybrid zone sites. For each tree, 10 terminal buds of different aspects and heights were categorized using negative values (–2 or –1) to indicate various degrees of bud dormancy, a 0 value to indicate budbreak, and positive values to in-

dicate the number of leaves on the young shoot. A tree's phenological state was the average of these 10 values. This phenological survey was repeated throughout the season.

Second, to document the response of beetles to staggered host phenologies, adults were censused in the hybrid zone on immature trees whose phenological state was determined using the above method. Censuses were standardized to 2 min/tree and repeated in mid-April ( $n = 100$  trees) and mid-May ( $n = 60$  trees). Most if not all individuals on a tree were censused in the 2-min period (range 0–94 beetles/2 min). Counts were subsequently normalized as  $\log(x + 1)$  and data analyzed to obtain a correlation between beetle density and tree phenology.

To determine if host shifting increased beetle fitness, adults were collected at one site in the hybrid zone within 1 wk of overwintering emergence and used in four treatments in a common garden. Each treatment consisted of eight sleeve cages (approximately 24 cm in height by 15 cm in diameter) with three pairs of beetles per cage. Cages were made of fine netting stretched over an internal wire frame. These treatments were: (1) beetles caged on Fremont, (2) beetles caged on narrowleaf, (3) beetles caged initially on narrowleaf, then shifted 2 wk later to Fremont, (4) beetles caged initially on narrowleaf, shifted 2 wk later to a hybrid host, then shifted again in 3 wk to Fremont. Cages were moved between host categories in response to the timing of budbreak. All cages were moved weekly, if not to a new host, then to a new branch of the same tree. This reduced possible cage effects on foliage and ensured adequate food for beetles. Treatments 1 and 2 were to assess beetle fecundity in Fremont and narrowleaf zones. Treatments 3 and 4 were to assess fecundity in the hybrid zone where host shifts are possible. The number of eggs laid and beetle mortality were recorded weekly. Beetles were not replaced and data were collected until all adults were dead. Lifetime fecundity was measured as average number of eggs laid per female per cage. Values from cages in the same treatment were then averaged to obtain a mean and standard error.

Production of leaf tissue on treatment trees was followed during the experiment to relate host phenology with beetle fecundity. Leaf tissue production was based upon the average number of new leaves initiated in a given period on a terminal shoot ( $n = 30$  shoots) times the average area of leaves at maturity ( $n = 100$  leaves).

To document the consistency in the timing of spring budbreak between tree genotypes, the phenology of trees in a common garden was compared with the phenologies of the parent trees from which the common garden trees had been cloned 10 yr before. The common garden was 32 km from the parental clones and  $\approx 100$  m higher in elevation. Leaf phenology was based upon an average of 50 buds categorized using the scale described earlier in this subsection.

### Experimental test of the hybrid sink hypothesis

To test if beetle fitness was greater on hybrid hosts relative to pure host genotypes, fitness parameters were measured in two experiments for larvae and adults reared on either pure or hybrid trees of the same age growing in a common garden.

Larval growth rates and mortality, and newly emerged adult dry masses were measured for individuals reared on Fremont (one genotype), narrowleaf (two genotypes), F1 type (two genotypes), or complex backcross (eight genotypes) hosts to assess the effect of host category on these parameters. Trees were classified using DNA analyses (Keim et al. 1989). Parameters were measured using five clutches of 10 newly eclosed larvae transferred onto each tree. Because larval clutches were not all transferred onto trees on the same day, larval growth rate was measured using degree-days to standardize for air temperature differences between transfer dates. Degree-day values (i.e., [(daily maximum temperature + daily minimum temperature)/2] - 10°C) were calculated and then summed to obtain a cumulative value as a measure of the time to pupation for each larva. Mean values for each clutch were used in analyses for between-tree differences. Each clutch was reared in a sleeve cage with cages moved within trees every 2–3 d. Moving cages ensured an abundance of food for larvae and reduced potential cage effects on foliage quality.

To assess the effect of host category on adult fecundity, lifetime fecundity was determined for adults feeding on either pure or hybrid hosts. Beetles were caught at the same time and site in the hybrid zone within 2 wk of overwintering emergence. Three pairs of beetles were placed in a sleeve cage with five such cages on one each of Fremont, F1 type, backcross and narrowleaf trees. Cages were moved weekly within trees. Weekly ovipositions were counted until all adults were dead. Lifetime fecundity was measured as the average number of eggs laid per female per cage. Values from cages in the same treatment were then averaged to obtain a mean and standard error. The effect of male absence on subsequent female fecundity in cages where all males had died was not examined but assumed to be equal across host categories.

### Experimental test of the distributional pulse hypothesis

A concentration of beetles in the hybrid zone could be attributed to a “pulse” in their distribution, i.e., beetles appear first at low elevations and then at progressively higher elevations as the season advances. This pattern could be due to colder temperatures delaying the emergence of overwintering beetles at higher elevations or delaying budbreak of cottonwoods at these sites. If the latter, beetles would not overwinter at high elevations but move to them in response to later host phenology. Regardless of the mechanism, the observed

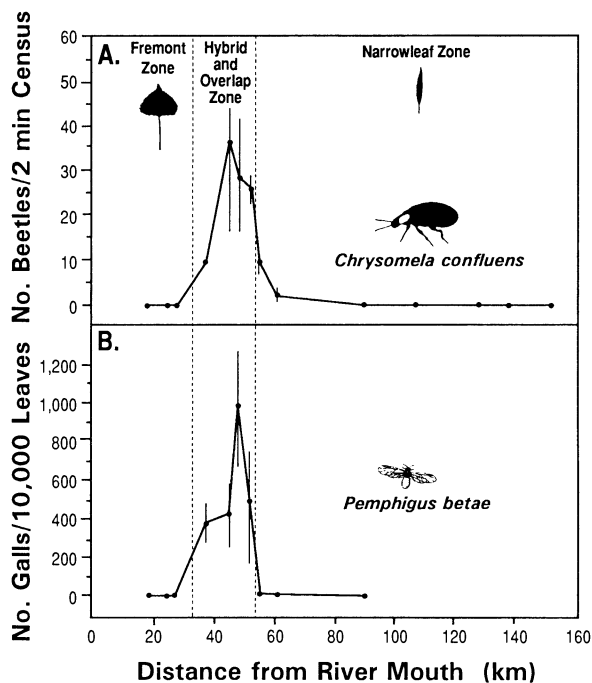


FIG. 1. (A) Over a 3-yr period, 91.1, 90.2, and 96.8% of the population of the oligophagist beetle *Chrysomela confluens* remained centered in the hybrid zone, which represents <3% of its host population. Values are 3-yr means ( $\pm 1$  SE) based upon 10, 2-min censuses per site. (B) The distribution of the specialist insect, the galling aphid *Pemphigus betae*, is remarkably similar to that of *C. confluens* (adapted from Whitham 1989).

pattern of distribution could be explained as a “pulse” in beetle abundance passing through the hybrid zone at the time of sampling.

This hypothesis was tested by repeating beetle surveys in 1990 to see if the concentration of beetles in the hybrid zone was seasonally stable. Beetles were censused from early spring until mid-June using the same 14 sites and the methods discussed previously.

## RESULTS

### Beetle distribution

For 3 yr, the distribution of *Chrysomela confluens* remained centered in the hybrid zone (Fig. 1A). Based upon mean site averages for Fremont, hybrid and narrowleaf zones, 91.1, 90.2, and 96.8% of the population in 1989, 1990, and 1991, respectively, was found in this zone. At the end of April 1991 when beetles were most abundant during the 3-yr study, mean site averages ( $\pm 1$  SE) for beetles counted per 2-min census in Fremont, hybrid and narrowleaf zones were  $0 \pm 0$ ,  $39.9 \pm 15.9$ , and  $1.3 \pm 0.9$  beetles, respectively. The distribution of this free-feeding beetle was remarkably similar to that of the specialist gall-forming aphid, *Pemphigus betae* (Fig. 1B) from which the hybrid sink hypothesis was developed.

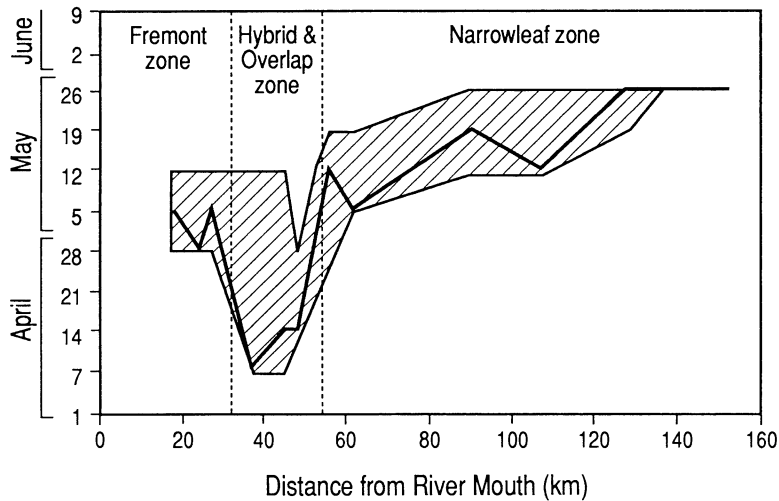


FIG. 2. Time of budbreak throughout the Weber drainage. The earliest availability of food for beetles emerging in the spring is in the hybrid zone where trees flush, on average, 2–7 wk ahead of trees in the pure Fremont and narrowleaf zones. (— time of spring budbreak based on the average phenologies of 10 trees at each site. Shaded area indicates the range between earliest and latest time of budbreak for individual trees at each site.)

#### Tests of hypotheses

*Phenological sink hypothesis.*—Four lines of evidence support this hypothesis. First, the hybrid zone provides the earliest source of food for beetles emerging in the spring and their subsequent offspring. Budbreak of narrowleaf and hybrid trees in the hybrid zone is 1–7 wk before trees in the narrowleaf zone and 3–5 wk before Fremont trees in hybrid and pure zones (Fig. 2). For example, in a spring survey of trees in the hybrid zone, 80% of Fremont trees ( $n = 25$ ) had not broken bud dormancy but only 4% of sympatric hybrid trees ( $n = 75$ ) were in a similar state. The remaining 96% of hybrid trees had an average of  $1.4 \pm 0.2$  leaves per

shoot. (Note: Because phenologies of sympatric, DNA-identified narrowleaf and hybrid trees were similar, and because pure narrowleaf trees are relatively rare in the hybrid zone [Keim et al. 1989], the two groups were collectively termed “hybrids” in this study unless otherwise stated.)

Second, the hybrid zone produces leaf tissue for *C. confluens* for a longer period than do pure zones. For example, narrowleaf trees provide an initial food source but Fremont provides a much greater source of new leaf tissue as leaf production on narrowleaf declines (Fig. 3). Between 19 and 27 May on adjacent trees in a common garden, Fremont flushed 4.43 leaves/shoot whereas narrowleaf cottonwood flushed only 0.63 leaves/shoot. Fremont leaves were 10-fold larger at maturity than narrowleaf leaves ( $44.2 \pm 1.6$  vs.  $4.4 \pm 0.1$  cm<sup>2</sup> leaves, respectively). Thus, Fremont shoots initiated what became 70-fold more leaf tissue for herbivorous insects than did adjacent narrowleaf shoots in the same period. Because trees in the hybrid zone have leaf phenologies and leaf sizes that span the entire range between pure narrowleaf and pure Fremont cottonwood, the hybrid zone provides a continuum of resources not available in adjacent pure zones.

Third, beetle density is correlated with the availability of new leaves. Censuses in the hybrid zone showed that beetle distribution in mid-April was correlated with host phenology ( $r^2 = 0.40$ ,  $n = 100$  trees; Fig. 4). Negatively scored trees (pre-budbreak) had significantly fewer beetles than did trees scored as 0 and above ( $9.71 \pm 1.45$  beetles/tree [ $n = 45$  trees] vs.  $34.81 \pm 3.26$  beetles/tree [ $n = 55$  trees], respectively).

In early spring, beetles did not avoid Fremont trees but rather were attracted to hybrid trees where food was available. Since Fremont trees have a later phe-

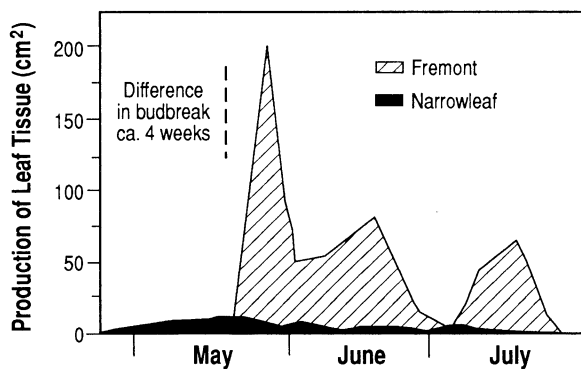


FIG. 3. Although narrowleaf trees flush 4 wk before adjacent Fremont trees, Fremont provides a much greater source of new leaf tissue for beetles at a time when the foliage on narrowleaf is mostly mature and, therefore, of reduced quality (see *Methods: The system* for references). (Values based on number of new leaves initiated by a shoot [ $n = 30$  shoots] in a given period times the average area of leaves [ $n = 100$  leaves] at maturity.)

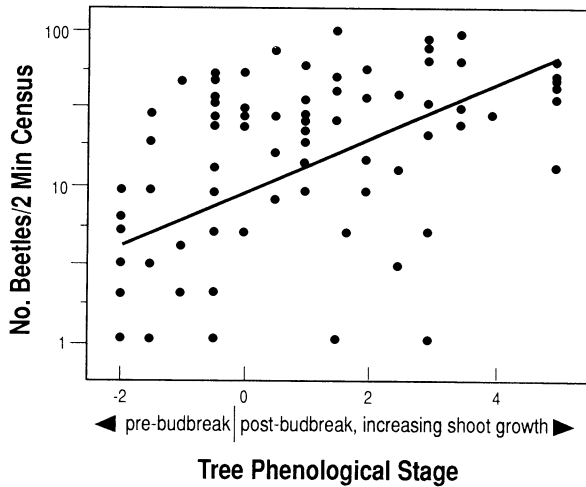


FIG. 4. In spring, beetles are more attracted to cottonwoods that have broken bud (phenological stage [PS] = 0 or above) than to trees that have not yet done so (PS < 0). Plotted above are actual numbers of beetles counted per tree during a 2-min census. When these counts are transformed as  $\log(x + 1)$  to normalize the data, the correlation is  $r^2 = 0.40$ ,  $Y = 0.94X + 0.17$ , and  $n = 100$  trees.

nology than sympatric hybrid trees, greater beetle densities on more phenologically advanced trees could be interpreted as an avoidance of Fremont hosts. However, when tree phenology was held constant (i.e., hybrids with late budbreak were compared to Fremont cottonwoods), there was no significant difference in beetle abundance on hybrid or Fremont hosts (hybrid:  $2.23 \pm 0.50$  beetles/tree,  $n = 30$  trees; Fremont:  $2.79 \pm 0.97$  beetles/tree,  $n = 30$  trees;  $P = .61$ ,  $t$  test).

The dramatic shift by the adult *C. confluens* population in late spring from hybrid hosts onto Fremont hosts is further evidence for the beetles' preference for young leaves (Fig. 5). In mid-April, almost 100% of the observed beetle population occurred on hybrid trees,

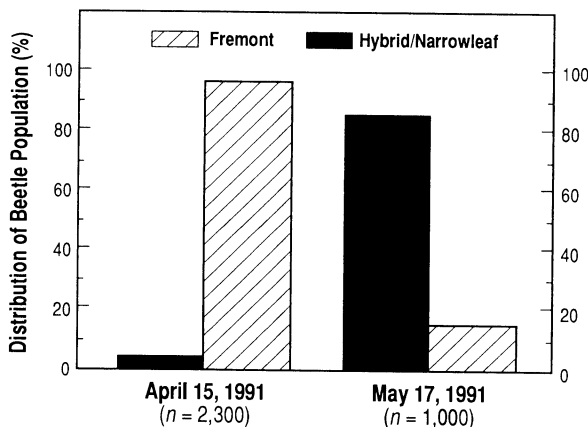


FIG. 5. In spring, almost 100% of the beetle population is on hybrid hosts but the population shifts onto Fremont by mid-May.

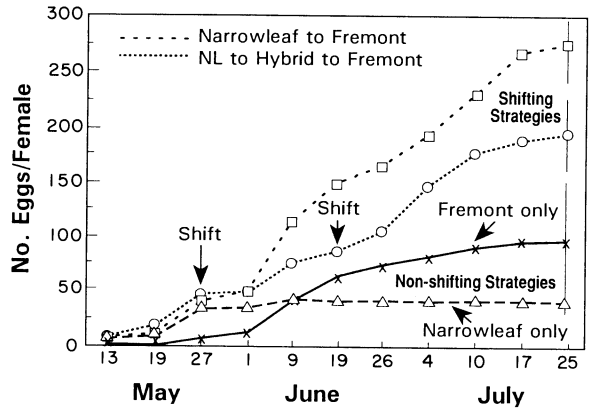


FIG. 6. In experimental comparisons of switching and nonswitching feeding strategies, the strategy that results in the highest beetle fecundity is to begin feeding on narrowleaf in the spring but then to shift onto Fremont later in the summer. Fecundity was significantly greater for beetles switched directly from narrowleaf to Fremont than for beetles switched indirectly from narrowleaf to Fremont via hybrids. However, both switching strategies significantly increased fecundity over that of beetles that remained caged on either Fremont or narrowleaf. (Standard error bars are shown for 25 July.)

which were then the only hosts flushing leaves. However, by mid-May, most of the foliage on hybrid trees had matured and over 80% of adults were now found on Fremont hosts which had only recently begun to flush leaves.

Fourth, cage experiments support the importance of expanded host phenologies in affecting beetle performance. Fecundity of beetles that were shifted from narrowleaf to Fremont cottonwood increased up to six-fold relative to controls (Fig. 6). Beetles restricted to narrowleaf trees laid an average of  $41.9 \pm 9.0$  eggs but the average fecundity of beetles that were shifted from narrowleaf directly onto Fremont hosts in midseason was  $276.3 \pm 35.4$  eggs. Beetles restricted to Fremont or shifted from narrowleaf to Fremont via hybrids had intermediate fecundities. The low average fecundity of females on Fremont was likely due to the high mortality of beetles before budbreak on these trees.

The *C. confluens* population likely remains concentrated in the hybrid zone year after year since the pattern of beetle distribution is closely tied to tree phenology, which is ultimately based upon host genotype. For example, the correlation for leaf flush of trees in 1982 with leaf flush of their derivative clones in 1991 was  $r^2 = 0.81$  (Fig. 7), arguing for a large genetic component to tree phenology. This correlation is particularly impressive given the separation in time and distance of parental trees from their clones. Further, although a genetic basis for phenological differences between species may be intuitive, the present correlation uses more closely related plants (i.e., two narrowleaf and six complex backcross hybrids [Keim et al. 1989]).

*Hybrid sink hypothesis.*—The hypothesis that *C.*

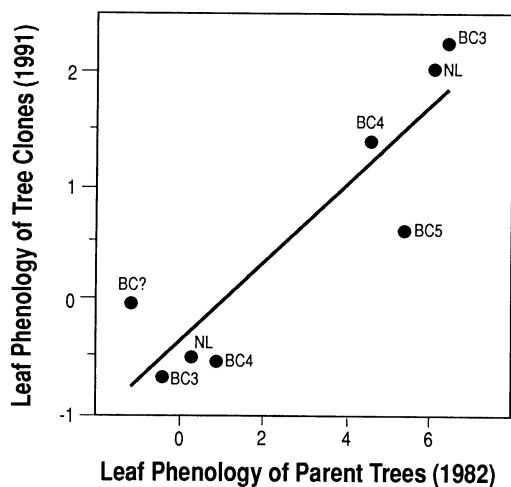


FIG. 7. The high correlation of leaf flush between trees in 1982 and their derivative clones in 1991 ( $r^2 = 0.81$ ,  $Y = 0.35X - 0.38$ ,  $n = 8$  tree genotypes) shows that the timing of spring budbreak is determined largely by tree genotype (BC = Backcross hybrid, NL = pure Narrowleaf). Leaf phenology was based upon an average of 50 buds categorized using the scale described in *Methods: Experimental test of the phenological sink hypothesis*

*confluens* had greater fitness on hybrid trees than on pure trees was not supported. There were no significant differences between host categories in larval mortality or dry masses of male or female beetles (Table 1). Only larval growth rate was significantly different between host categories, in which case larvae reared on narrowleaf took longer to reach pupation than did larvae reared on Fremont, F1 type, or complex backcross trees. Further, in decreasing order, lifetime fecundity was greatest on Fremont, then on complex backcross, F1 type, and narrowleaf hosts with significant differences between each group (Fig. 8). Although these results do not eliminate this hypothesis, due to the small number of host genotypes available for testing, they indicate that relative to phenology, the potential role of greater fitness on hybrid hosts would be a minimal contribution in affecting beetle distribution.

*Distributional pulse hypothesis.*—Beetle distribution was seasonally stable. Although appearing earlier in the spring at lower elevations than at higher elevations,

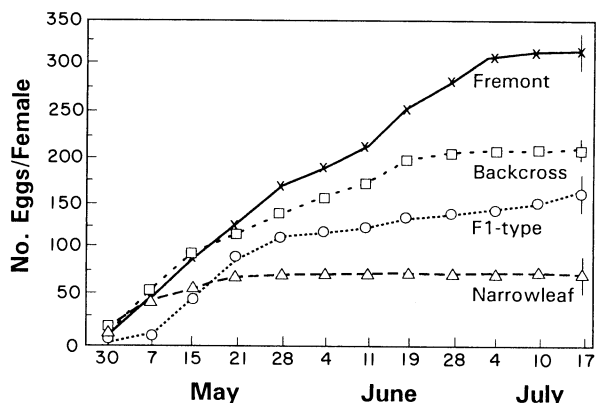


FIG. 8. When beetles are restricted to one host type, fitness, in terms of lifetime fecundity, is not greatest on F1 or backcross hybrids as might be predicted by the hybrid sink hypothesis. (Standard error bars shown for 17 July are based on cage averages.)

only small numbers of beetles were observed outside the hybrid zone throughout the summer. This pattern was consistent for each of four surveys conducted from mid-April to mid-June 1990 (Fig. 9).

## DISCUSSION

### *The phenological sink hypothesis*

First leaf flush and expanded host phenology best explain the concentration of *C. confluens* in the hybrid zone. We call this the phenological sink hypothesis and describe it in detail below.

*First flush.*—Early leaf flush in the hybrid zone selects against the establishment of beetle populations in pure zones. Leaf flush on Fremont lags 3–5 wk behind beetle emergence at the same site. Thus, beetles that emerge in, or migrate to, the Fremont zone in early spring likely starve before ovipositing, e.g., in one experiment, 83% ( $n = 48$ ) of beetles caged on Fremont before leaf flush died in a 2-wk period. This is also true at the higher elevations of the narrowleaf zone. Potentially, beetles could delay emergence in, or migration to, pure zones to coincide with budbreak but this would delay the onset of oviposition 2–7 wk (Fig. 2). Since females oviposit for up to 10 wk (Figs. 6 and 8), synchronous emergence with budbreak in pure zones would

TABLE 1. None of three measures of fitness indicates that the performance of *Chrysomela confluens* is significantly greater on hybrid cottonwoods than on pure Fremont and narrowleaf trees. (Values are means  $\pm$  1 SE.) Different superscript letters within columns indicate significant differences;  $P < .05$ .

Host type	Sample size*	Number of trees	Growth rate (cumulative degree-days†)	Percent larval mortality	Adult dry mass (mg)	
					Male	Female
Fremont	5	1	161.34 $\pm$ 3.62 <sup>a</sup>	18.00 $\pm$ 9.70 <sup>a</sup>	7.55 $\pm$ 0.67 <sup>a</sup>	9.65 $\pm$ 0.84 <sup>a</sup>
F1 hybrid	10	2	166.37 $\pm$ 2.11 <sup>a</sup>	6.00 $\pm$ 3.40 <sup>a</sup>	7.59 $\pm$ 0.36 <sup>a</sup>	10.04 $\pm$ 0.48 <sup>a</sup>
Backcross	40	8	166.08 $\pm$ 1.18 <sup>a</sup>	9.25 $\pm$ 2.01 <sup>a</sup>	8.18 $\pm$ 0.18 <sup>a</sup>	10.46 $\pm$ 0.29 <sup>a</sup>
Narrowleaf	10	2	175.22 $\pm$ 3.94 <sup>b</sup>	12.00 $\pm$ 4.42 <sup>a</sup>	7.15 $\pm$ 0.44 <sup>a</sup>	9.20 $\pm$ 0.69 <sup>a</sup>

\* Number of larval clutches (each initially containing 10 individuals) from which values are derived.

† Degree-day = [(daily maximum temp + daily minimum temp)/2] - 10°C.

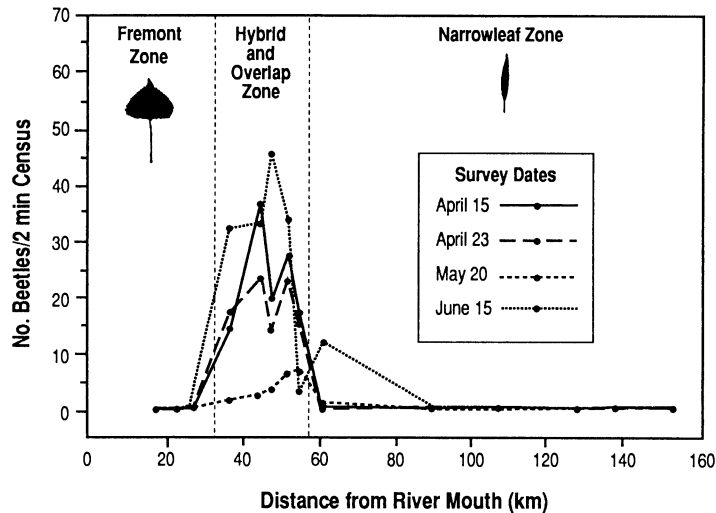


FIG. 9. Because the pattern of beetle distribution is seasonally stable (Fig. 9), the concentration of beetles in the hybrid zone cannot be attributed to an artifact of sampling time. (Values are beetle counts per 2-min census, averaged for 10 censuses/site.)

significantly reduce the time available for oviposition, subsequent larval development, and feeding by immature adults preparatory to winter diapause.

*Expanded host phenology.*—Staggered host phenology allows *C. confluens* to increase its fitness in the hybrid zone. Chrysomelids attain greatest fitness on young leaves relative to mature leaves (see *Methods: The system*). Hybrid trees provide the earliest source of young foliage in the spring and, as these leaves mature, Fremont begins to flush. Beetles prolong their period of feeding on young leaves by shifting from hybrid trees onto sympatric Fremont at this time (Fig. 5). Beetles do not move into the Fremont zone to find young leaves (Fig. 9), presumably because the presence of Fremont trees in the hybrid zone makes it unnecessary. Other benefits of this shift have not yet been studied but females that oviposit on different host species over time reduce the density of their siblings, which could reduce sibling competition and lower predation/parasitism rates on their progeny.

In summary, conditions intrinsic to the hybrid zone act as a phenological sink resulting in high densities of *C. confluens*. The hybrid zone provides both the earliest sites for feeding (i.e., first flush) and also the longest availability of young leaves (due to an expanded phenology), which combine to increase beetle density in this zone. Similarly, highly synchronous emergence of the overwintering herbivore population with host budbreak and the subsequent presence of new foliage have been associated with outbreaks of other insect species (Morrow 1983 and references within, Hunter 1991).

#### *Alternative hypotheses not supported or tested*

Neither of the two other hypotheses examined explained the concentration of *C. confluens* in the hybrid zone. The hybrid sink hypothesis was rejected because,

unlike *P. betae* (Whitham 1989), *C. confluens* did not have greater fitness on hybrid hosts relative to pure hosts. In other systems, however, hybrid susceptibility may still explain why organisms are concentrated in hybrid, rather than pure, zones, of their hosts (e.g., cestodes and nematodes of mice, Sage et al. 1986; insects and fungi on *Eucalyptus*, Drake 1981, Whitham et al. 1991). The distributional pulse hypothesis was rejected because the pattern of beetle distribution was seasonally stable (Fig. 9) and, therefore, could not be attributed to an artifact of sampling time.

How plant chemistry affects *C. confluens* distribution was not examined. Salicaceous plants such as *Populus* contain phenolic compounds that influence chrysomelid distribution (Smiley et al. 1985, Tahvanainen et al. 1985, Denno et al. 1990, Soetens et al. 1991). Further, chrysomelids, including *C. confluens*, sequester by-products of these compounds for chemical defense against natural enemies (Kearsley and Whitham 1993 and references within). Thus, the concentration of *C. confluens* in the hybrid zone could be due to differences in plant chemistry making hybrid trees more attractive and/or providing a higher level of chemical protection, to beetles than pure cottonwoods (as per Denno et al. 1990). However, if hybrid trees are more attractive, why do beetles subsequently shift onto Fremont trees? Further, natural enemies of *C. confluens* (a parasitic pteromalid wasp and the pentatomid bug, *Podisus* sp.) appear concentrated in the hybrid zone where they cause high levels of mortality (K.D. Floate, unpublished data). We think it unlikely that plant chemistry or natural enemies explain the virtual absence of beetles from pure Fremont and narrowleaf zones. However, hybrid plant chemistry and its effects on herbivores remain an interesting aspect of insect-plant interactions that requires further study.



### Further considerations

The phenological sink hypothesis is distinct from the hybrid sink hypothesis (Whitham 1989) because the former probably does not require hybridization between hosts to promote the concentration of insects, merely disparate phenologies between sympatric host species. As the phenologies of sympatric narrowleaf and hybrid trees in our cottonwood system are similar, the removal of hybrid hosts may accentuate differences between phenologies of Fremont and narrowleaf trees but beetles should still be able to increase their fecundity by shifting between hosts. However, because host phenology is strongly correlated with plant genotype (Fig. 7), hybridization may be important in expanding the period of leaf flush in other hybrid plant systems.

The phenological sink may explain insect outbreaks on some introduced species or varieties of plants. For example, the cottonwood leaf beetle, *Chrysomela scripta*, is a major defoliator of *Populus* species and their hybrids in nurseries and plantations in eastern North America (Solomon et al. 1976, Caldbeck et al. 1978, Burkot and Benjamin 1979). In the current study, high densities of *C. confluens* were sustained by a prolonged period of leaf flush on *Populus* in the spring. New leaves are also produced during the summer but, as they represent a declining fraction of total foliage, and are typically consumed well before full expansion, they are a limited resource at this time. Because tree genotype largely determines the timing of budbreak within a site (Fig. 7), growing different varieties or species of *Populus* in a localized area will increase the genetic variation of this host population and may extend its period of leaf flush in the spring. If our observations on *C. confluens* are true for its congener, *C. scripta*, an extended spring flush would be predicted to increase *C. scripta* populations. In summary, increasing the genetic variation of an insect's host population can create conditions conducive to an outbreak of the insect as per the phenological sink hypothesis.

Because two distinct mechanisms, hybrid susceptibility (Whitham 1989) and expanded leaf flush, have now been documented that result in plant hybrid zones providing unique habitat for insects and being centers for their distributions, arguments for studying and conserving these zones become even stronger. For example, the elimination of an *Eucalyptus* hybrid zone in Australia could potentially remove the several insect species nearly restricted to this zone from the system (Whitham et al. 1991). Hybrid zones also represent habitat for insect communities that is distinct from that of adjacent pure zones of the parental plant species. Insects may be more common (Drake 1981, Whitham et al. 1991), equally abundant (Floate 1992), rarer (Boecklen and Spellenberg 1990), or intermediate (Manley and Fowler 1969, Roskam and van Uffelen 1981, Aguilar and Boecklen 1992) in hybrid zones relative to pure zones. Finally, 30–80% of all plant species

may have derived from hybridization events (Stace 1987, Wendel et al. 1991). Consequently, by conserving hybrid zones, it can be argued that focal points of biodiversity, distinct insect communities, and important centers of plant evolution are preserved (Whitham et al. 1991, Floate 1992).

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